

ABSTRACTS

1. PRODUCTION OF FLOCCULI IN MEDIA CONTAINING
PABA OR PROCAIN AND YELLOW PIGMENT IN
PAS-MEDIUM BY CULTURE OF ACID-FAST BACILLI

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Received for publication, Dec. 1, 1965.

Several species of acid-fast bacilli were observed to produce flocculent precipitates when cultivated in liquid media containing PABA or procain, and some species produced a bright yellow pigment in liquid culture media containing PAS. Some species gave rise to both these phenomena.

These phenomena seem to be useful for the classification of acid-fast bacilli in combination with Wayne's test and PAS-black test.

2. ANALYTICAL STUDIES ON TUBERCULIN REACTION.

Part 1. EFFECT ON SIX FACTORS ON TUBERCULIN REACTION

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Received for publication, Nov. 1, 1965.

For the purpose of clarifying the effect of six factors on tuberculin reaction, the following experiments were carried out on guinea pigs.

1) Skin testing was carried out with o-Aminophenol Azo-Tuberculin (AT) on three groups of animals sensitized with liquid paraffin containing 0.2, 1 and 5 mg dry weight of heat-killed tubercle bacilli, respectively.

No difference in the development of skin hypersensitivity was observed among the groups.

2) Tuberculin skin hypersensitivity developed several days after the sensitization, grew in intensity rapidly for two weeks and more slowly thereafter till the end of sixth week, and then gradually subsided.

3) AT prepared from heated culture filtrate was a little more potent in producing skin reaction than AT from unheated culture filtrate, but AT's prepared from many different lots of each strain, heated or unheated culture filtrate, are observed to be quite equal in potency.

4) Skin tests carried out on H₃₇Rv-sensitized animals with AT's made from seven strains of acid-fast bacilli revealed that AT from H₃₇Rv was the most potent and was followed by AT's from Ushi-10, Kofu, P-1, Sanbayashi and Yamamoto-S in that order.

This order is considered to reflect the degree of taxonomic kinship of the above-mentioned strains.

5) Equal quantities of AT in unequal concentrations were observed to elicit skin reactions of unequal intensities.

With small quantities of AT, the skin reaction produced was more intense when the concentration was highest, but with large quantities of AT the reaction was more intense when the concentration was lower.

6) Tuberculin hypersensitivity was observed to be higher and more uniform in young animals (350 gm body weight) than in old ones (600 gm or more body weight).

3. FUNDAMENTAL STUDIES IN CHEMOTHERAPY OF TUBERCULOSIS.

PART 70. *IN VITRO* ANTITUBERCULOUS ACTION OF PASCON

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Received for publication, Dec. 3, 1965.

The tuberculostatic action of PASCON, which is a compound of Scormine and PAS, against various strains of human tubercle bacillus when used alone and in combination with SM and INH, was studied, as well as the concentration of the drug in the blood of human subjects after oral administration and the *in vitro* acquisition of resistance to it by tubercle bacilli. The results obtained were as follows :

1) The growth inhibitory action of PASCON to 15 strains isolated from patients with pulmonary tuberculosis and H₃₇Rv were almost equal to or slightly lower than PAS. Complete cross resistance was observed between PAS and PASCON, but none between PASCON and INH or SM.

2) The blood concentrations of PASCON in human beings were approximately 57/ml and 37/ml three hours and five hours respectively after oral administration of 100 mg of PASCON.

3) The effects of repeated *in vitro* exposure to PASCON+SM or PASCON+INH on the development of drug resistance of H₃₇Rv strain and change of tuberculostatic

power of the drugs were observed. a) PASCON+SM was effective for retarding the appearance of PASCON- or SM-resistant tubercle bacilli. PASCON+INH, however, was effective for retarding the appearance of PASCON-resistant bacilli, but not of INH-resistant bacilli. b) In successive cultivation of H₃₇Rv in Dubos medium containing PASCON+SM or PASCON+INH, PASCON and SM, or INH showed no synergistic effects of tuberculostatic activities, at first cultivation, but noticeable effects after the appearance of the drug resistant bacilli in the subsequent cultivations.

4. PROPERTY OF AZO-PROTEIN TO CAUSE DELAYED TYPE SKIN REACTION

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Received for publication, Nov. 9, 1965.

Guinea pigs sensitized with hen egg albumin (HEA) in complete or incomplete Freund's type adjuvant clearly developed delayed skin reaction to HEA from day 3 or 4 and immediate skin reaction from day 9 or 10. After the appearance of the immediate skin reaction, the delayed component was difficult to evaluate because of the preceding immediate component. However, the animals sensitized with HEA in complete adjuvant (FCA) showed demonstrable, though slight, post-arthus induration and erythema, after the skin testing with HEA. These persistent skin reactions probably represent the delayed hypersensitivity. The authors, who had the experience of finding o-aminophenol carrying tuberculoprotein, in spite of its lack of *in vitro* antigenicity, to be as active as tuberculoprotein in causing delayed skin reaction, carried out some experiments concerning the antigenicity of various azo-HEAs for skin reaction, and it was clearly demonstrated that animals sensitized with HEA in FCA retained delayed type skin hypersensitivities against azo-HEAs after the appearance of IHS against HEA, but those sensitized with HEA in FIA did not.

Thus, it has been proved that azo-HEAs are useful tools for examining the delayed skin reaction in animals showing IHS to HEA.

5. EFFECT OF FREUND'S TYPE ADJUVANT ON DEVELOPMENT OF DELAYED TYPE SKIN HYPERSENSITIVITY

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Received for publication, Nov. 9, 1965.

Experiments were carried out on four groups of guinea pigs for the purpose of clarifying the effect of Freund's type adjuvant with and without mycobacteria (FCA and FIA, respectively) on the development of delayed skin hypersensitivity.

- Group 1. Animals injected into the hind foot pads with hen egg albumin (HEA) emulsified with FCA.
- Group 2. Animals injected into the hind foot pads with HEA emulsified with FIA.
- Group 3. Animals injected with FCA into the hind foot pads and with HEA by various other routes,
- Group 4. Animals injected with FIA into the hind foot pads and with HEA into the hind legs.

The results obtained were as follows :

1. The Groups 1 and 2 developed delayed skin response with negative cornea reaction (Jones-Mote type DHS) to HEA on the 3rd or 4th day and immediate response (IHS) on the 9th or 10th day after the sensitization. Even after the development of IHS, the existence of tuberculin type DHS was clearly demonstrated in Group 1, but not in Group 2, by means of intradermal injection of azo-HEA and cornea test.
2. Group 3 developed DHS and IHS in the same manner as Group 1 when HEA was injected into the hind thighs, but hardly any DHS when it was injected into the forelegs or back skin or intracardially.
3. The animals of Group 4 developed no DHS.

Thus, two possible explanations for the development of 3 kinds of skin reaction have been offered. One explanation is that FCA stimulates the proliferation of numerous types of stem cells and the antigen selects from among them those types respectively responsible for Jones-Mote type DHS, tuberculin type DHS and IHS. The second is that FCA causes a common ancestor cell to differentiate into three types, which then are stimulated by the antigen to proliferate further,

6. EXPERIMENTAL ANTICANCER STUDIES.

PART 29. RELATION BETWEEN ANTICANCER ACTIVITY OF HEMOLYTIC STREPTOCOCCI AND KIND OF THEIR SUSPENDING MEDIA

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I. Effect of the presence of RNase-core and penicillin in the preincubation medium of hemolytic streptococci on their anticancer activity

A 20-fold concentrate of hemolytic streptococci grown in either 0.8% RNase-core broth or plain broth was used as the original cocci-suspension. Using the basal medium (BM), which was composed of 675mg maltose, 6ml 20% KH_2PO_4 (adjusted to pH 6.9 with NaOH), 12ml 2% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and 66ml distilled water, following three kinds of media were prepared : RNase-core (5mg/ml) containing BM, penicillin (4.1×10^4 units/ml) containing BM, and BM containing both RNase-core (5mg/ml) and penicillin (4.1×10^4 units/ml). With each of these three media and BM, a series of diluted cocci-suspensions were prepared and incubated at 37°C for 20 minutes. Anticancer experiments were then performed with each of these four series of diluted cocci-suspensions.

The results obtained may be summarized as follows :

1) Penicillin-pretreated cocci-suspension was far effective in depriving Ehrlich ascites carcinoma cells of their invading power to mice, as compared with the streptococcal suspension, which had not been received penicillin pretreatment. As reported previously, it was also seen from the data of present work that after penicillin-pretreatment, the anticancer activity of hemolytic streptococci grown in 0.8% RNase-core broth is markedly greater than that of the organisms grown in plain broth.

2) Pretreatment of streptococcal suspensions in the presence of RNase-core (5mg/ml) at 37°C for 20 minutes did not cause an appreciable enhancement in their anticancer activity. On the contrary, a marked reduction in the anticancer activity was observed in the experiments with streptococcal suspensions pretreated in the presence of both penicillin and RNase-core at 37°C for 20 minutes.

II. Relation between anticancer activity of hemolytic streptococci and kind of their suspending media, carried out comparatively in the presence or absence of penicillin

Hemolytic streptococci grown in 0.8% RNase-core broth were used throughout.

The diluted cocci-suspensions were prepared with following different media :

- 1) inactivated whole serum (rabbits) containing penicillin (4.1×10^4 units/ml) or none,
- 2) 0.1% RNase-core BM containing penicillin or none, and
- 3) BM containing penicillin (4.1×10^4 units/ml) or none.

All cocci-suspensions were incubated at 37°C for 20 minutes. Anticancer experiments with thus treated cocci-suspensions were performed with following order of anticancer activity results :

Suspension of cocci in BM containing penicillin > Suspension of cocci in serum containing penicillin > Suspension of cocci in 0.1% RNase-core BM containing penicillin »
 [Suspension of cocci in BM \geq Suspension of cocci in serum \geq Suspension of cocci in 0.1% RNase-core BM].

7. STUDIES ON THE PHENOMENON OF HIGH PROMOTION BY NUCLEIC ACID OF THE PRODUCTION OF STREPTOLYSIN-S OF HEMOLYTIC STREPTOCOCCI.

Part 23. INEFFECTIVENESS OF TRYPAN BLUE ON THE HEMOLYTIC ACTIVITY OF STREPTOLYSIN-O

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Received for publication, July 19, 1965.

Data are presented to show that trypan blue does not exhibit any antagonistic activity on the Streptolysin-O hemolysis, and that trypan blue is very effective in inhibiting the hemolysis caused by Weld's hemotoxin (Serum extract of hemolytic streptococci), as is in the case of Streptolysin-S.

8. EXPERIMENTAL ANTICANCER STUDIES.

PART 28. EFFECT OF LIVING HEMOLYTIC STREPTOCOCCI ON VARIOUS TRANSPLANTABLE ASCITES TUMOR CELLS IN *IN VITRO*-*IN VIVO* ASSAY SYSTEM

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As a continuation of our anticancer studies with hemolytic streptococci, a series of the experiments were carried out to investigate whether living streptococci are capable of eliciting anticancer activity against various kinds of transplantable ascites tumor cells, employing the so-called *in vitro-in vivo* assay method. The ascites tumors used in the present experiments were three mouse tumors, Ehrlich carcinoma, Sarcoma 180, Leukemia SN 36, and three rat tumors, Yoshida sarcoma, and ascites hepatomas AH 130 and AH 66.

The principal results obtained were as follows :

1) Regardless of the differences in the kinds of the animal tumors, living hemolytic streptococci caused a definite inhibition of invasion power of the tumors in experimental animals.

Additionally, an appreciable amount of streptolysin S was detected in the supernatant fluids of the six incubation mixtures of living streptococci and tumor cells.

2) In these respects, heat-killed streptococci were, however, entirely without effect.

9. LACTOBACILLUS, STAPHYLOCOCCUS AND CANDIDA DERIVED FROM ORAL CAVITY.

PART 1. THE RATES OF DETECTION AND THE BIOLOGICAL PROPERTIES OF THE ISOLATED BACTERIA

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Received for publication, Sep. 30, 1965.

The author examined the gargling water from 125 normal school children (SCW) and from 58 patients being treated for tuberculosis (TBW) and 50 specimens of carious teeth (CT), for the lactobacillus, staphylococcus and candida they contained, and compared the rates of their occurrence and the biological properties of isolated strains.

The results obtained are as follows.

1) All the three species of the bacteria were found in lower proportions of SCW than TBW and CT. This was especially conspicuous about lactobacillus and candida. Almost the same percentage of TBW and CT contained lactobacillus, but staphylococcus

and candida were found in higher proportions of CT and TBW, respectively.

2) Simultaneous presence of two or three species of the bacteria was more frequent in TBW and CT than in SCW. It was especially noteworthy that candida was always accompanied by either lactobacillus or staphylococcus in CT.

3) The lower the pH of the gargling water was, the larger was the proportion in which lactobacillus and candida were detected. But there was no correlation recognized between the pH and the presence of staphylococcus.

4) There was no marked difference between the staphylococci from TBW and SCW in their biological properties, but drug resistance was observed in a larger proportion of the strains from TBW than those from SCW.

10. LACTOBACILLUS, STAPHYLOCOCCUS AND CANDIDA DERIVED FROM ORAL CAVITY.

PART 2. MIXED CULTURES OF LACTOBACILLUS AND STAPHYLOCOCCUS AND CANDIDA

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Received for publication, Dec. 17, 1965.

Mixed cultivation of lactobacillus and staphylococcus with candida was carried out and the growth of the bacterial population was compared with the corresponding growth in the pure culture. The results obtained are as follows:

1) When cultured with candida, lactobacillus shows a growth curve almost identical in the logarithmic growth stage with that shown by simple culture. But in the death stage the lowering of population curve is less sharp in the mixed culture than in the pure culture. This phenomenon takes place also when the culture filtrate of candida is added to the medium in which lactobacillus is cultivated by itself. The active factor contained in the filtrate responsible for the phenomenon is found dialysable.

On the other hand the growth of candida is restrained by the presence of lactobacillus, probably owing to the action of the latter to lower the pH of the medium.

2) Staphylococcus cultured with candida shows temporary lowering of the population after the maximum has been reached. But it reaches a minimum on the second day and goes up again thereafter. The phenomenon is shown almost equally by both coagulase-positive and -negative cocci. When staphylococcus is inoculated in a medium containing no other nitrogen source than ammonium sulphate, it failed to grow at all, but if the culture filtrate of candida is added to the medium, normal growth is seen

to proceed. The factor responsible for this phenomenon is also dialyzable.

On the other hand the candida shows an decrease when grown in mixed culture with coagulase-negative strain of staphylococcus, but indifferent to the presence of coagulase-positive strain.

11. A STATISTIC STUDY ON ORGAN-METASTASIS OF PULMONARY CANCER

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In recent years, cases of primary lung cancer are noticeably increasing. The cause of lethality is considered to consist, to some extent, in metastasis formation in the important organs.

Autopsy was performed in 650 cases during the period of 5 years from 1959 to 1963 in Kanazawa University. Neoplasm was found in 281 cases pathologic anatomically, 43 cases of which were diagnosed as primary lung cancer.

Statistic analysis was made on these 43 cases from the standpoint of hematogenous and lymphogenous metastasis in the distant organs.

Among these 43, males were 34 and females were 9, respectively corresponding to 80 and 20 per cent.

Most patients dying of pulmonary cancer belonged to the older age group, almost 80 per cent being in the fifth and sixth decades.

Among these autopsy cases of primary lung cancer, adenocarcinoma had the highest frequency of metastasis formation and squamous carcinoma had the smallest frequency. It is assumed that operative treatment is most favorably indicated in cases of squamous carcinoma.

12. A REVIEW OF SURGICAL TREATMENT OF PULMONARY CARCINOMA

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Received for publication, Dec. 10, 1965.

Experience with 28 cases of primary pulmonary carcinoma treated at Toyama Prefectural Central Hospital in three years (1962-1965) is reviewed, with particular reference to surgical treatments and their results.

Among the total of 28 cases, radical operation was performed in seven (pneumectomy in six and lobectomy in one) and the tumor was found nonresectable in four owing to invasive infiltration and metastasis.

The remaining 17 cases were of advanced carcinoma and diagnosed inoperable when first seen.

Moreover, from the standpoint of tumor-host relationship, ligation of the splenic artery was carried out in cases of advanced and inoperable carcinoma with a view to activating the function of the R. E. S.. The effect of the ligation and some other information obtained are reported.

13. A CASE OF BRONCHIAL ADENOMA TURNING MALIGNANT

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A case of bronchial adenoma was reported on a 43 year-old housewife. Her chief

complaints were headache and stiffness in the right shoulder.

The chest X-ray showed an isolated round tumorous shadow in the right lower field which had reportedly been noticed for two years.

Right lower lobectomy was performed on June 4, 1965 and the tumor was diagnosed histopathologically as a bronchial adenoma with partial malignant transformation. There was no metastasis in the hilar or mediastinal peribronchial lymph nodes. The postoperative course was uneventful and the patient is doing well.

A clinicopathological review was made on the collected literature here and abroad as to the incidence, location, growth patterns, signs and X-ray findings of the bronchial adenoma.

14. A CASE OF PULMONARY ABSCESS

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Received for publication, Dec. 10, 1965.

We treated a case of pulmonary abscess with moderate anemia and lowered lung function rate.

The patient, a 37 year old housewife, was admitted to the Kitagata Rinkoyen Sanatorium on Nov. 6, 1961. She was chronically ill with marked irritant cough and copious sputum (about 230 ml daily), low pulmonary function rate (V.C. 960 ml 36%), moderate anemia (r.b.c. count 267×10^4 , w.b.c. count 14,800, Sali'hemoglobin test rate 70%) and marked rise of erythrocyte sedimentation rate (110 mm in one hour and 128 mm in two hours).

Her chief complaints were marked cough and sputum of one year duration.

After admission to our clinic, the treatment slightly improved the blood condition (r.b.c. count 307×10^4) and pulmonary function rate (V.C. 1,400 ml, 53%) but had no effect on the irritant cough, sputum (about 200-230 ml daily) and chest X-ray. Left lower lobectomy was tried on Nov. 21, 1962, one year after admission but had to be given up owing to low blood pressure fall (Max. 78 mmHg).

After that she had a slight change for the better in the general state but her cough and sputum did not become abated.

Reoperation was performed four months after the first operation.

The portal entry to lung abscess in the left lower lobe was allowed to remain as an open wound on the left side chest and was treated with antibiotic and conservative therapy.

After some days her complaints were reduced and the putrid discharge from the wound stopped about eight months after reoperation and the wound was healed up. Her inflammatory and general conditions improved. The patient resumed her regular work in March 1964, one year after reoperation. Her erythrocyte sedimentation rate was 5 mm in one hour and 17 mm in two hours on her release from our sanatorium.

Publications not appearing in the Ann. Rep. Tbc.

Kanazawa (1965)

Okamoto, H., Shoin, S., Koshimura, S., and Shimizu, R. :

Experimental Anticancer Studies. Part XXVII. Effect of Penicillin Treatment of Hemolytic Streptococci, Grown in RNase-Core Broth, on Their Anticancer Activity. Japan. J. Exp. Med., **35**, 249-254, 1965.;

The *in vitro* pretreatment with penicillin of hemolytic streptococci grown in 0.8% RNase-core broth caused a marked increase in the ability to deprive Ehrlich ascites carcinoma cells of their invading power in mice.

The four streptococcal suspensions were examined quantitatively for their anticancer capacity with following order ; penicillin-pretreated suspension of cocci grown in 0.8% RNase-core broth > penicillin-pretreated suspension of cocci grown in plain broth > suspension of cocci grown in 0.8% RNase-core broth > suspension of cocci grown in plain broth.