

ABSTRACTS

—Original—

1. A HEMAGGLUTINATION PHENOMENON BETWEEN TANNED
ERYTHROCYTES AND THE SERUM OF GUINEA PIGS.

PART 5. THE EFFECT OF CHEMOTHERAPEUTIC TREATMENT ON THE TANNED
ERYTHROCYTE-AGGLUTINATING ACTIVITY OF THE SERUM OF
GUINEA PIGS IN EXPERIMENTAL TUBERCULOSIS

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Eighty guinea pigs were inoculated subcutaneously with 0.1 mg of H37Rv tubercle bacilli. Twenty-six days after inoculation, the animals were divided into five groups as follows: (1) untreated controls, (2) treated subcutaneously with a daily dose of 8 mg of dihydrostreptomycin, (3) treated subcutaneously with a daily dose of 1 mg of isoniazid, (4) treated subcutaneously with a daily dose of 10 mg of kanamycin, and (5) treated orally with a daily dose of 0.2 gm of p-aminosalicylate. The medication was continued for 82 days. The animals were bled by heart puncture and then sacrificed between 118 and 125 days after infection. The extent of tuberculous lesions in the spleen, lung and liver was examined macroscopically. Sera were tested for their tanned erythrocyte (TE)-agglutinating activity by the method described previously.

The results obtained were as follows: The gross tuberculous lesions found in the animals of the three groups which had been treated respectively with dihydrostreptomycin, isoniazid and kanamycin were much less extensive than those found in the untreated control group. In addition, the TE-agglutinating activity of the sera of these treated animals was strikingly reduced as compared with a high degree of the activity in the control animals. By contrast, the animals of the p-aminosalicylate-treated group hardly differed from the control animals in the degree of the visceral tuberculous lesions as well as in the serum activity.

2. STUDIES ON O-AMINOPHENOL AZO-TUBERCULIN.

PART 25. IN VIVO ANTIGENICITY OF O-AMINOPHENOL AZO-TUBERCULIN

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o-Aminophenol azo-tuberculin (OA-Azo-T) is prepared by adding diazotized o-aminophenol to old tuberculin obtained from Sauton's synthetic medium and acidifying the mixture with HCl to pH 4.8. It is generally accepted that the OA-Azo-T thus prepared is stable in its potency of inducing skin reaction and free from undesirable side effects on living bodies.

Another important property for good tuberculin to have is freedom from antibody-producing power. In the present study, rabbits were injected with the preparation by various means for the purpose of evaluating the in vivo antigenicity of OA-Azo-T, and the power of their sera to agglutinate OA-Azo-T-sensitized red cells was determined. Since OA-Azo-T has hemolytic property the conditions for preparing OA-Azo-T-sensitized red cells were examined first of all, and then the passive hemagglutination test was carried out.

The results obtained show that OA-Azo-T is far less antigenic than the protein fraction obtained from old tuberculin (PF) in producing the antibody against PF and is completely inactive in producing the antibody against o-aminophenol.

3. STUDIES ON ATYPICAL ACID-FAST BACILLI.

PART 3. TYPE-SPECIFICITY OF O-AMINOPHENOL AZO-TUBERCULIN (AT)
IN SKIN REACTION

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Skin tests with OT or AT made from various acid-fast bacilli were carried out on guinea pigs sensitized with heat-killed bacilli. While the OT-s did not show type-specificity in skin reaction except for *M. fortuitum*, the AT-s showed it clearly for human tubercle bacilli, photochromogens, nonphotochromogens, scotochromogens and *M. fortuitum*.

Furthermore the strains of *M. fortuitum* were classified into 3 groups according to their AT-specificity in skin reaction, and two of them showed PAS-black reaction on media containing PAS.

Thus, combined employment of Wayne's test, PAS-black test and AT-skin test is considered to be useful for classification of acid-fast bacilli, especially *M. fortuitum*.

4. ANALYTIC STUDIES ON TUBERCULIN REACTION.

(PRELIMINARY REPORT)

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In order to study the tuberculin reaction analytically, experiments concerning the tuberculin skin reaction were carried out on guinea pigs sensitised with heat-killed bacilli ($H_{37}R_v$ and P 1) and a tuberculin reactivity curve was obtained by plotting the size of erythema against the tuberculin-concentration. In this preliminary report are outlined the significance and application of the curve.

5. STUDIES ON THE METABOLISM OF LIPID IN TUBERCULOSIS.

PART 3. PATTERN OF THE CHOLESTEROL CHANGE IN SERUM AND
IN THE LUNG AND LIVER IN EXPERIMENTAL
TUBERCULOSIS OF RABBIT

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Three groups of rabbits were employed in order to measure the quantity of cholesterol in serum, and in the lung and liver tissues in experimental tuberculosis produced by modified Yamamura's method.

Group 1. Rabbits sensitized with heat-killed bovine tubercle bacilli five times in a course of 3 weeks.

Group 2. Rabbits sensitized in the same way and infected with live bovine tubercle bacilli

1 week after the last sensitization.

Group 3. Untreated as control.

The quantity of cholesterol was measured in Groups 1 and 2 on the 10th, 20th, 40th, 50th and 60th days after the last sensitization.

The results obtained were as follows:

1. The serum cholesterol level was higher in rabbits of Groups 1 and 2 than in those of Group 3 in all the period of testing, being highest on the 20th day in Group 1 and on the 30th day in Group 2, and then decreased gradually.

2. The cholesterol level in the lung tissue was higher in Group 1 than in Group 3 in the early stage after the sensitization. But the level in Group 1 began to decrease from the 30th day and eventually became lower than that in Group 3.

On the other hand, the cholesterol level in the lung tissue was much lower in Group 2 than in Group 3 in the early stage. The level in Group 2 began to increase from the 30th day but always remained lower than that in Group 3.

3. The pattern of cholesterol change was almost the same in the liver tissue as in the lung tissue.

6. EXPERIMENTAL ANTICANCER STUDIES.

PART 24. EFFECT OF BIS (2-HYDROXY-3,5-DIBROMOPHENYLAZO)- 4-N-PROPYLPHLOROGLUCINOL [AZO-106] ON THE TUMOR INDUCTION BY 3,4-BENZOPYRENE IN MICE

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In our previous anticancer studies, it has been shown by Hirata et al. that bis(2-hydroxy-3,5-dibromophenylazo)-4-n-propylphloroglucinol, Azo-106, was effective in inhibiting the growth of Ehrlich carcinoma in mice, and that such an anticancer effect was also expected with Azo-106 in experiments, in which some other transplantable ascites tumors, Sarcoma 180, Yoshida sarcoma, and lymphatic leukemia SN 36, were used.

The present study was designed to investigate influence of Azo-106, in comparing with that of trypan blue [TB], on the tumor induction by 3,4-benzopyrene [BP] in mice.

The principal results obtained are as follows :

1) Long-term administration of Azo-106 was proved to be not toxic for normal mice; even after twice-a-week intraperitoneal injections of an Azo-106 dose of 0.3 mg (1/3 LD₅₀)

for the period of 18 weeks, there observed neither degenerative nor neoplastic cells in visceral organs, i. e. liver, spleen, kidney and lung.

2) Inhibitory effect of Azo-106 on the induction of tumor by BP in mice was clearly demonstrated in the experiments, in which mice received simultaneously subcutaneous applications of BP (a single dose of 0.25 mg BP per a week for 5 weeks) and intraperitoneal administrations of Azo-106 (a single dose of 0.3 mg, once or twice a week, for 16 weeks).

The rate of tumor incidence in group of mice treated with Azo-106 was 25 per cent (average weight of the tumors = 2.4 gm), while that of untreated control group 73 per cent (average weight of the tumors = 3.4 gm).

By contrary, the experiments of TB treatment (31 repeated intraperitoneal administrations, twice a week, of a single dose of 0.2 mg of the dye) gave entirely negative results in these respects.

3) Additionally, all the tumors formed at the BP injection site of experimental animals showed histologically characteristic picture of fibrosarcoma.

7. EXPERIMENTAL ANTICANCER STUDIES.

PART 26. INEFFECTIVENESS OF STREPTOCOCCAL STRAIN HAVING STREPTOLYSIN O PRODUCING ABILITY ONLY

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A series of experiments designed to examine the factors influencing the streptolysin S producing ability and cancer-cell destructive ability of hemolytic streptococci have indicated that there may exist a close relation between these two abilities.

Thus, our experimental anticancer study with hemolytic streptococci is now being conducted on the supposition that the RNA-effect, i. e., interaction of streptolysin S synthesizing enzyme(s) of the cocci and RNA contained in tumor cells, underlies the phenomenal manifestation of destruction of tumor cells.

The question of whether or not the ability to produce streptolysin O of the cocci may take part or contribute in exerting their anticancer effect remained thus far unanswered.

In the present work, following five strains of group A streptococci were investigated from the point of view of the anticancer activity :

a) C203S, Sa and Su strains, which are capable of producing both streptolysins S and O.

- b) Blackmore strain, known to produce streptolysin-S only.
- c) C203U strain, known to produce streptolysin O only.

The method of anticancer experiments with these strains, was the same as that employed in previous studies.

The results obtained were as follows :

- 1) The strains having the ability to produce streptolysin S and O, C203S, Sa and Su, all were tested to be effective in depriving the invasion power of Ehrlich ascites carcinoma cells to mice.
- 2) A comparable anticancer effect was also observed in the Blackmore strain which produces only streptolysin S.
- 3) However, the C203U strain, which produces streptolysin O but not streptolysin S, was tested to be ineffective in this respect.

8. STUDIES ON THE PHENOMENON OF HIGH PROMOTION BY NUCLEIC ACID OF THE PRODUCTION OF STREPTOLYSIN S OF HEMOLYTIC STREPTOCOCCUS

PART 24. ON THE STREPTOLYSIN S INDUCING EFFECT OF RNA PREPARATIONS
ISOLATED FROM LIVERS OF SEVERAL ANIMAL SPECIES AND
FROM DIFFERENT TUMOR CELLS

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Employing the phenol method described by Kirby, ribonucleic acid (RNA) preparations were isolated from normal livers of six Mammalian genera (mouse, guinea pig, rat, rabbit, pig and beef) and from regenerating rat liver on the one hand, and from six different kind of transplantable rodent tumor cells (Ehrlich ascites carcinoma, Sarcoma 180, lymphatic Leukemia SN36, Yoshida sarcoma, AH 130 and AH 66) and also from two human carcinoma tissues on the other.

These RNA preparations were tested for their streptolysin S inducing activity.

The principal results obtained were as follows :

- 1) Experiments carried out by employing the culture method.

It was found in experiments, in which ordinary broth containing 1% RNA preparation to be tested was inoculated with hemolytic streptococci and incubated at 37° for 40 hours, that all the preparations of RNA were very active in inducing streptolysin S formation.

To be noted is the fact that the activity of the RNA preparations was approximately the same regardless of whether the preparation derived from Mammalian livers or tumor

cells.

- 2) Experiments carried out by employing the resting cell system method
 - a) It was found in the experiment, in which the RNA preparation to be tested was incorporated in Bernheimer's basal medium, that the RNA preparation from normal rat liver was far less active in inducing streptolysin S formation by resting streptococci than any other RNA preparations.
 - b) Furthermore, treatment with pancreatic ribonuclease (RNase) of the RNA preparation from normal rat liver caused rather a decrease in its streptolysin S inducing activity. This was more clearly demonstrated in experiment with RNase-core sample isolated from RNase digest of the RNA preparation.

In other words, the results were somewhat complicated by the finding that the RNA (or RNase-core thereof) from normal rat liver caused only a little activity, when tested in the resting cell system medium, while the same preparation, when tested by culture method, was as highly active as the RNA preparations of all other sources.

At present, it is impossible to offer any satisfactory explanation for this exceptional case.
- 3) Data of determination experiments on the base composition of all the RNA preparations were also presented.

9. FUNDAMENTAL AND CLINICAL STUDIES IN CHEMOTHERAPY OF DYSENTERY WITH NALIDIXIC ACID (WINTOMYLON)

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A series of experiments was carried out concerning the antibacterial potency of wintomylon (WM), concentrations of the drug in blood and urine after oral administration, and the therapeutic effect of the drug on patients with dysentery. The results obtained were as follows :

- 1) The antibacterial potency of WM for *Shigella* was observed to be almost equal to that of streptomycin and gabbromicina, but inferior to T-ran which is a nitrofurantoin derivative.
- 2) In vitro experiments showed that *Sh. sonnei* and *E. coli*, readily acquired resistance to WM, but no cross resistance to the other drug tested.
- 3) A large amount of the drug was rapidly excreted in urine, but an effective blood level of WM was demonstrably maintained till 4 hours after oral use.

4) In the treatment of patients having *Sh. sonnei*, single use of WM was effective for improvement of symptoms and for negativation of the bacilli in feces, but combined therapy with KM was more effective.

5) WM produced no undesirable side effect.

Thus, WM is considered to be an excellent drug for treatment of dysentery due to *Shigella* resistant to number of drugs.

10. ELECTROCARDIOGRAPHIC FINDINGS IN GENERAL ANESTHESIA

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The recent progress of surgery is largely due to improved advancement of anesthesia, wider use of blood transfusion and the advent of potent antibiotics. The operative procedure has been done under the extensive indication, even being operated upon the patients who were in the poor risk because of various functional disorders.

In performing general anesthesia, it is very important to assess correctly the surgical risk of the patient. The most significant one among the factors which cause the poor risk of the patient, is impediment of the cardiac function, since it is closely related to the patient's death. The present authors have studied the functional disorders of the heart in general anesthesia by means of electrocardiographic examination.

The present authors' results revealed that bundle-branch block and myocardial damage were pointed out as the essential factors to cause the risk. However, their studies led to the conclusion that the risk could be correctly evaluated only by taking into consideration of many factors, such as the electrocardiographic findings, the age of the patient and amount of the surgical intervention.

11. COMPLICATIONS OF GENERAL ANESTHESIA

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The authors have studied the complications which occurred in 2588 cases of general anesthesia performed for 5 years from 1960 to 1964. The patients who had been in poor

risk before the operation, were often suffering from fall of blood pressure, arrhythmia or protracted apnoea during anesthesia. For preventing these complications, it is important to spend sufficient time for improving the patient's general condition before the operation and to administer cardiac agents and transfusion in adequate amounts during the operation.

In the aged or new-born, the complication following anesthesia often proved fatal. Therefore, the surgeons should cooperate with the anesthetists in making timely efforts for preventing complications before, during and after the operation, so as to minimize fatalities due to anesthesia.

12. METASTATIC CARCINOMA OF THE PITUITARY POSTERIOR LOBE AND THE INFUNDIBULUM..... AN AUTOPSY REPORT

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Metastatic carcinoma of the posterior lobe of the pituitary and the infundibulum noted in a case of gastric carcinoma which clinically manifested the symptoms of diabetes insipidus. A fifty-four-year-old Japanese male had been complaining about severe thirst and polyuria with progressing epigastric pain and weight loss for about two months prior to admission in November, 1964 when he was diagnosed as carcinoma of stomach. A drinking of 4 to 5 litre of water and up to 3 litre of urinary output per day had been noted. The specific gravity of the one day urine ranged from 1.010 to 1.030. An exploratory laparotomy revealed carcinoma of stomach at the pyloric region extending beyond the serosa with further infiltration into the head of pancreas and gastrojejunostomy was performed. Seven days after the operation, the patient developed jaundice which gradually increased in the severity up to his death on January 18, 1965. Autopsy disclosed the posterior lobe of pituitary being completely replaced by metastatic carcinoma of partly signet-ring type cells which extending through the stalk to the infundibulum region (Fig. 2, 3 and 4). The periphery of the metastatic lesion of the infundibulum showed a zone of yellowish-green color on the unfixed fresh cut surface which revealed negative for iron by hemosiderin staining on the histologic slides and considered to be bile deposits. It was estimated that the mechanism of the jaundice of the brain tissue on this adult case was due to the focal destruction of the blood-brain-barrier by the tumor tissue. The jaundice of the case was due to an extrahepatic biliary duct obstruction by the infiltrating gastric tumor.

According to Willis, in almost all cases of metastatic tumors to the pituitary, the tumors occupy the posterior lobe and deposits in the anterior lobe are exceedingly rare. The primary tumors of these reported cases were of the breast, lung, pancreas, thyroid and prostate in the decreasing order and this case was of the stomach origin. The presenting symptoms of diabetes insipidus on this case was apparently attributed to the metastatic lesion of the pituitary or the infundibulum or both. The kidney showed no evidence of changes which might be able to explain as to the cause of the excessive urination of the patient.