Abstracts

1. BIOCHEMICAL STUDY OF THE STREPTOLYSIN-S INDUCING EFFECT OF RIBONUCLEIC ACID : A REVIEW

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Contents :

2. INFLUENCE OF ANTITUBERCULOUS AGENTS ON TUBERCULIN PRODUCTION OF TUBERCLE BACILLUS

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Studies were made to ascertain whether the growth-inhibiting action of antituberculous agents exerted any influence upon the tuberculin production of growing cultures of a human tubercle bacillus, Aoyama B strain.

The bacilli were grown on Sauton's medium. The antituberculous agents tested were streptomycin, isonicotinic acid hydrazide, kanamycin, p-aminosalicylic acid and o-aminophenol. A number of flasks, each containing 50 ml of Sauton's medium, were inoculated with the tubercle bacilli and incubated at 37°C. After about two weeks' incubation, when the cultures had grown all over the surface of the fluid, the flasks were divided into lots of three each. One lot of them was further incubated without addition of drugs (control I lot). Another lot was immediately subjected to preparation of tuberculin as is described below (control II lot). The others were further incubated after addition of various amounts of the antituberculous agents to be tested. Two or three weeks after the addition of drugs, each of the lots, including the control I lot and the drug-containing lots, was subjected to preparation of tuberculin as follows : the culture fluid in each flask was freed of the bacilli by decantation and filtration through a Seitz filter (culture filtrate). The bacilli left after the removal of culture fluid were throughly washed with sterile water and then immersed in 20 ml of 0.1 M citrate solution, followed by incubation at 37°C overnight. After the incubation the citrate solution was passed through a Seitz filter (citrate-tuberculin). Finally, the bacilli were killed by heat and weighed after drying. Two series of tuberculin samples thus obtained from the the respective lots were assayed for their skin-reacting potency upon guinea pigs infected with a virulent human tubercle bacillus, Nagahama strain.

The results of comparative skin tests with the tuberculin samples from the control lots and with those from the drug-containing lots are summed up as follows :

1. The skin-reacting activity of culture filtrates increased progressively even if the growth of the tubercle bacilli was completely interrupted by the addition of any one of the five antituberculous agents, their activity being occasionally comparable to that of the control I lot. It was also shown that, when added to the cultures together with streptomycin, neither chloramphenicol nor 8-azaguanine affected the tuberculin production of the tubercle bacilli inhibited by streptomycin.

   It is to be added here that the five antituberculous agents used markedly differed from one another in the growth-inhibiting activity against the bacilli grown on Sauton's medium, while they exhibited the same order of the activity in Kirchner's medium.

2. The skin-reacting activity of citrate-tuberculin samples was proportional to the yields of the bacilli; thus, the samples obtained from the lots in which the growth of the bacilli was inhibited by the drugs were less potent than that from the control I lot, but nearly equal to that from the control II lot.

3. IMMUNOLOGICAL STUDIES ON SENSITIZED ERYTHROCYTES

Part 16. The Antigenicity of the Fractions from Typhoid Bacilli and the Red Cells Sensitized with the Fractions

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Comparative studies were made of the in vivo and in vitro antigenicity of protein and polysaccharide fractions from typhoid bacillary bodies by intravenously injecting to rabbits individually the fractions and red cells sensitized with them. The resuts obtained showed the polysaccharide fraction to be higher in the in vivo anigenicity than the protein fraction, but lower in the in vitro antigenicity.

4. STUDIES ON THE LIPID-METABOLISM IN EXPERIMENTAL PULMONARY EDEMA

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The authors have studied the lipid-metabolism in experimental pulmonary edema for several years, using kurono and Sakai's circular paper chromatography.

The results obtained were as follows :

Unsaturated fatty acids such as oleic and linoleic acids, and saturated fatty acids such as miristric and palmitinic acids were found in the liver- and lung-tissues of the experimentally produced pulmonary edema. There was no essential difference between a normal dog and an edematous dog, in the fatty acids found in the lung- and liver-tissues. However, the unsaturated fatty acids showed a transient increase in the early stage of the pulmonary edema and gradually decreased with the lapse of time.

5. EXPERIMENTAL ANTICANCER STUDIES

Part 15. Anti-tumor Activity of Bis-azoamylphloroglucinol Derivatives and Other Hydroxyazo-compounds on Ehrlich Ascites Carcinoma in Mice

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In the course of our experimental anti-cancer studies attempted to search a potent anti-tumor agent in the series of 2,2'-dihydroxyazobenzene, evidence has been presented to show that five homologues of bis (2-hydroxy-3,5-dibromo-phenylazo)- alkylphloroglucinols are effective to inhibit the growth of Ehrlich ascites carcinoma in mice, and that among them propyl-compound (AZO-106) was especially effective against Sarcoma 180, Yoshida sarcoma and mouse leukemia SN 36.

For extending this line of work, 10 bis-azoamylphloroglucinol derivatives and 7 related hydroxyazo-compounds were newly synthesized and tested for their effect against Ehrlich ascites carcinoma in mice.

As the results of a comparative experiment, it was demonstrated that bis-azoamylphloroglucinol derivatives having halogen atom (Cl or Br) in the benzene nucleus were effective in causing a prolongation of the life of experimental animals, and that bis-azoamylphloroglucinols having either $-NO_2$, or $-CH_3$, or -COOH, or $-AsO(OH)_2$ in the benzene nucleus of azo-component were all found to be without effect.

Thus, it may be said that the existence of halo-o-diazophenol as azo-component in these hydroxyazo derivatives is necessary for represent anti-tumor effect.

6. EXPERIMENTAL ANTICANCER STUDIES

Part 17. Carcinostatic Activity of (2-Hydroxy-3,5-dibromophenylazo)-phloroglucinol, -pyrazolone and -imidazole Derivatives on Ehrlich Ascites Carcinoma in Mice

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Continuing the previous work, thirty-five azo-compounds including (2-hydroxy-3,5-dibromophenylazo)-phloroglucinol, -pyrazolone and -imidazole derivatives were thereafter synthesized, and tested for their anti-cancer effect on Ehrlich ascites carcinoma in mice.

The results of these anti-cancer experiments showed that none of azo-derivatives which have acylalkylphloroglucinol as coupler component was effective in causing the life-span of the experimental animals bearing Ehrlich ascites carcinoma, and of thirteen azo-derivatives, which have dialkylphloroglucinol as coupler component, only 6-(2'-hydroxy-3',5'-dibromophenylazo)-2,4-dipropylphloroglucinol was found to be slightly effective.

It is to be noted that azopyrazolone derivatives tested were found to be without effect, while of five azoimidazole derivatives three compounds were tested effectively.

Publications not appearing in the Ann. Rep. Tbc. Kanazawa (1961)

Ohtaki, T. : *In vivo* Fate of o-Aminophenol. III. Experiments on o-Aminophenol Conjugates in Urine of Rabbits Following Oral Administration of o-Aminophenol. *Yakugaku Zasshi*, **81**, 53, 1961.

o-Aminophenol was given orally to a rabbit and Substances in the urine originating from the aminophenol were examined. The presence of o-aminophenol, o-aminophenyl glucosiduronic acid, o-aminophenyl hydrogensulfate, and o-acetamidophenol were confirmed by individual isolation. 1-o-Hydroxyanilino-1-deoxyglucuronic acid was not isolated directly but its presence was proved indirectly, while neither o-hydroxyphenylsulfamic acid nor 3-amino-phenoxazine-2-one was detected. Quantitative examination of these substances in the urine after oral administration of 1 gm of o-aminophenol showed that approximately 7.6% of the administered aminophenol was excreted unchanged, 26% as o-aminophenyl glucosiduronic acid, 18% as o-aminophenyl hydrogensulfate, and 1.2% as o-acetamidophenol.

Ohtaki, T. : *In vivo* Fate of o-Aminophenol. IV. Experiments in Man and in Rabbit on the Relationship Between Quantity of o-Aminophenol Given Orally and that of Conjugated Sulfuric Acid and of o-Conjugated Glucuronic Acid Excreted in Urine. *Yakugaku Zasshi*, **81**, 139, 1961.

Several dosages of o-aminophenol were administered orally to human subjects and rabbits, and the amounts of conjugated glucuronic acid and sulfuric acid excreted in the urine were examined. It was found that a living organism had the ability to change o-aminophenol into O-glucuronide and ethereal sulfate according to the amount of o-aminophenol administered, but detoxication by glucuronide was found to take place preferentially over that of ethereal sulfate.

Koshimura, S. and Shoin, S. : Experimental Anticancer Studies. Part 13. On the Streptolysin-S-Synthetizing and Anticancer Activities of Cell-Free Extract from Living Hemolytic Streptococci. *Gann*, **51**, 309, 1960.

Preparation of biologically active cell-free extract(CFE), as well as its acetone powder (EAP), has been achieved by grinding hemolytic streptococci with alumina under the controlled conditions of temperature and others. Both samples, CFE and EAP, were employed for streptolysin-S-synthetizing and anticancer studies, and the results of which were as follows :

1) When kept a mixture of EAP (as well as CFE) and yeast RNA at 37°C for 2 hours, appreciable amounts of streptolysin-S were produced.

2) Incubation of a mixture of EAP and Ehrlich ascites carcinoma cells at 37°C for 1.5 hours caused complete loss of the invasion power of the carcinoma cells to mice.

3) Treatment at 56°C for 30 minutes resulted in complete inactivation of the samples.

Shoin, S. : Experimental Anticancer Studies. Part 14. Anticancer Experiment with *Streptococcus hemolyticus* in Immunized Animals. *Jap. J. Pharmacol.*, **10**, 119, 1961.

1) The susceptibility of the mouse to Ehrlich carcinoma cells was not affected by the immune state of animals to hemolytic streptococci.

2) The anticancer effect of hemolytic streptococci was observed in animals immunized with streptococci, as was in normal non-immunized animals.

Koshimura, S., Hirata, R. and Shoin, S. : On the Streptolysin-S-Synthetizing and Anticancer Activities of Cell-Free Extract from Living Hemolytic Streptococci.

Cancer Chemotherapy Reports, **13**, 107, 1961.

Koshimura, S. : Malignant Tumor and Hemolytic Streptococcus.

Igaku-no-Ayumi, **39**(10), 551, 1961.