Abstracts

1. IMMUNOLOGICAL STUDIES ON SENSITIZED ERYTHROCYTES

PART 19. IMMUNOLOGICAL SIGNIFICANCE OF ERYTHROCYTES SENSITIZED WITH THE HEAT-EXTRACT OF SHIGELLA
No. 3. THE EXPERIMENTS UPON THE SERA FROM SHIGELLOSIS PATIENTS

SEKIYA YANAGI
Department of Bacteriology and Immunology, The Research Institute of Tuberculosis, Kanazawa University
(Director: Prof. Masamichi KAKISHITA)
Received for publication, June 15, 1963.

In the previous animal experiments, it was observed that hemagglutination and hemolysis reactions of the erythrocytes sensitized with the heat-extract of Shigella (E) were more sensitive and specific than their bacterial agglutination reaction. In the present clinical experiments the bacterial agglutination, hemagglutination, hemolysis and precipitation reaction tests were performed on 18 sera from shigellosis patients and as control on 35 sera from seemingly healthy persons.

The results obtained were as follows:

1) The bacterial agglutinin titer was lower than hemagglutinin titer in the patients' sera as well as in the control sera.

2) No hemolysis of E-sensitized red cells was caused by any of the test sera.

3) All the sera tested showed scarcely any positive precipitation reaction.

4) The hemagglutination titer of the patients' sera was higher than that of the control sera.

5) The absorption tests revealed that each patient's serum contained specific antibodies against the infecting strain.

6) The patients' sera and the control sera were observed to react with red cells sensitized with the heat-extract of Proteus OX_{19} (Ep) or of Escherichia coli (Ec), and the anti-Shigella antibody titer in the patients' sera was observed to decrease by absorption with Ep or Ec-sensitized red cells.

2. ON THE PHARMACOLOGICAL PROPERTIES OF BIS (2-HYDROXY-3,5-DIBROMOPHENYLAZO) -N-PROPYLPHLOROGLUCINOL (AZO-106)

ISAMU SATO
Department of Pharmacology, School of Medicine, Kanazawa University
(Director: Prof. Hajime OKAMOTO)
Received for publication, June 18, 1963.

Azo-106 has been shown by Hirata in 1957 to exert an anticancer activity in experimental

animals.

The purposes of the present report were to study the pharmacological properties of the compound, in comparion with some chemically or pharmacologically related drugs.

The followings are the principal results obtained so far:

I. Effect of Azo-106 on paramecia and isolated organs:

1) Azo-106 was toxic for paramecia, minimum concentration lethal to the organisms being 1:200,000.

2) The isolated frog heart responded to low concentration of Azo-106 (1:5,000) with a decrease in amplitude and rate of contraction.

3) The blood vessels of frogs were constricted by the perfusion of Azo-106 solution.

4) The tone and movements of isolated intestinal strips of guinea-pigs were inhibited with Azo-106.

5) Azo-106 caused first a transient stimulation and then relaxation of the isolated uterus strips of guinea-pigs.

6) Both propylphloroglucinol and phloroglucinol were tested to be almost inactive in any of above-mentioned experiments.

II. A moderate increase in the erythrocyte sedimentation rate was observed in rabbits received a single intravenous dose of 8 mg Azo-106 per kilogram bodyweight. But, no change was observed in blood clotting time in any of these animals.

III. The results obtained in the experiments, in which the effect of Azo-106 upon the blood picture of rabbits was studied, may be summarized as follows:

1) After a intravenous injection of 8 mg Azo-106/kg (which corresponds to 1/5 the LD_{50}) there occurred a moderate increase in the total white blood cell count due mainly to the appearance of lymphocytes. The rise in the white blood cell count could be observed even 3～4 days after the injection.

However, almost no change was observed either in the erythrocyte count or in the hemoglobin value.

2) More prolonged rise in the white blood cell count was observed in experiments, in which the animal received a single daily dose of 4 mg Azo-106 successively for 10 days. In these cases, a slight transient reduction in the hemoglobin and erythrocytes was accompanied.

3) Parallel experiments carried out with Thio-TEPA in dose of 2.5mg/kg (≒1/5 the LD_{50}) and mitomycin C in dose of 1 mg/kg (≒1/5 the LD_{50}) have shown that both anticancer agents caused a marked decrease in white blood cell count, accompanied by a slight reduction in hemoglobin and erythrocytes.

3. STREPTOLYSIN FORMATION BY RIBOSOMAL RNA OF *ESCHERICHIA COLI*

Yoriko TAKETO and Akira FUJIMURA

Department of Pharmacology, School of Medicine, Kanazawa University
(Director: Prof. Hajime OKAMOTO)

Received for publication, June 15, 1963.

The aim of the present study was to test the efficiency of ribosomal RNA of *E. coli* for the production of streptolysin S in resting cell system. The data indicated that ribosomal RNA (r-RNA) of *E. coli* was almost inactive in native state, but remarkable activity was observed after digestion by ribonuclease I. Some of the properties of streptolysin S formed in the presence of *E. coli* r-RNA core were also presented.

4. STUDIES ON BIOSYNTHESIS OF MONOIODOTYROSINE

II. The Mechanism of Enzymatic Iodination of Tyrosine

Ryozo WATANABE

Pathological Department, School of Medicine, Kanazawa University
(Director: Prof. Tachio ISHIKAWA)

Received for publication, July 1, 1963.

1. The mechanism of iodination of tyrosine was investigated by using the purified apoprotein and a cofactor.

2. The oxidation of iodide is carried out by the action of a cofactor.

3. Tyrosine is activated by the cofactor and the action of an apoprotein is necessary to combine the activated tyrosine and iodine.

4. It was found that oxygen is needed only in the reaction between the cofactor and tyrosine.

5. We could demonstrate the iodination of tyrosinyl group in protein with our soluble enzyme system. But high concentration of the protein exhibited on inhibitory action on the enzyme reaction.

5. STUDY ON SERUM LIPID IN INFANT NUTRITION

KYOICHI ASAI
Department of Pediatrics, School of Medicine, Kanazawa University
(Director: Prof. Ichiro SAGAWA)
Received for publication, July 1, 1963.

Author investigated the difference between breast and bottle feeding from the point of the levels of serum lipids (*e.g.* cholesterol, its fraction, lipoprotein, phospholipids and total lipids) in infants, and also the effect caused by linoleic acid on the serum lipids, using two kinds of milk fomula.

Author has concluded that in improvement of the diet fat in infants nutrition, it requires more circumspection to decide necessary content of E.F.A. because the factor relating fat metabolism in breast milk has not been solved sufficiently yet.

6. STUDIES ON THERAPEUTIC EFFECTS OF FURAN DERIVATIVES IN DYSENTERY

MASAMICHI KAKISHITA, TOMOO MATSUDA
AND SEKIYA YANAGI
Department of Bacteriology and Immunology, The Research Institute of Tuberculosis, Kanazawa University
(Director: Prof. Masamichi KAKISHITA)

ATSUYUKI SUGIBAYASHI, TAKESHI YOKOI
AND KENJI SHINTANI
Kanazawa Municipal Hospital
(Director: Dr. Kenzo YURI)
Received for publication, July 10, 1963.

Dysenteric patients were orally treated with four kinds of furan derivatives as follows.

Furazolidene, 0.2—0.4 mg daily to 22 cases.

Panfuran, 20—60 mg daily to 18 cases, including 5 carriers of dysentery bacillus.

3-Miranon-M, 0.8—1.0 gm daily to 22 cases, including 7 carriers of dysentery bacillus.

Miran, 60—120 mg daily to 21 cases, including 4 germ-carriers.

The results were as follows:

1) Among the four drugs miran showed the most marked antibacterial effect against drug-sensitive and drug-resistant strains of dysentery bacillus.

2) Every one of the four furan derivatives was found to have therapeutic effects on dysentery patients at least as potent as Kanamycin, Paromycin and Colimycin.

3) Each of the furan derivatives easily produced drug resistance in dysentery bacilli *in vitro*,

but no cross resistance against any other antibiotic unrelated to furan.

4) The drugs were found to be present in effective antiblastic concentrations in the patients' feces for four days following the termination of medication.

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

ISOLATION OF HIGH POTENCY STREPTOLYSIN-S PREPARATION FROM 1% RNASE-CORE BROTH CULTURE OF *Streptococcus Hemolyticus*

AKIRA FUJIMURA

Department of Pharmacology, School of Medicine, Kanazawa University
(*Director: Prof. Hajime OKAMOTO*)

Received for publication, July 22, 1963.

The purpose of present work was to establish a method for obtaining high potency streptolysin-S (St-S) preparation, which could be conveniently used either for performing further studies on the purification and chemical nature of the toxin or for the detection of cancer cells in circulating blood.

I. The principal results obtained in the preliminary experiments, in which the necessary conditions for maximal production by hemolytic streptococci of St-S in the media containing ribonucleic acid preparation were studied, were as follows:

1) Incubation at 37°C for 2~3 hours of a mixture [Bernheimer's basal medium (pH 7) containing Core II 0.6 mg/ml, 7ml + thick cocci suspension, 0.7 ml] resulted in a supernatant fluid exhibiting hemolytic activity up to a dilution of 1:32,000.

2) Incubation at 37°C for 30 (~48) hours of a 1% Core II broth (pH 7.6), after inoculating with hemolytic streptococci, resulted in a supernatant fluid exhibiting hemolytic activity up to dilutions of 1:256,000 (~512,000).

3) In this regard, both Cores I and II (29% and 40% alcohol-precipitable fractions from RNase I digest of commercial yeast ribonucleic acid, respectively) were most effective in producing high potency culture fluid, followed by Core II of regenerated RNA from Ag-RNA complex, commercial yeast RNA and Core III (50% alcohol-precipitable fraction), and regenerated RNA, in that order.

II. On the basis of these results, a method was developed for preparing a highly potent streptolysin-S preparation (C-INF_1 Fraction, minimum hemolytic concentration 1:100~200 millions) from 30-hour culture fluid of hemolytic streptococci grown on 1% Core II-broth.

The method comprises of two main courses as shown in Table 9:

1) Isolation of C-AI Fraction with minimum hemolytic concentration of 1:102,400,000.

2) Isolation of C-INF_1 Fraction with minimum hemolytic concentrations of 1:102,400,000~

204,800,000.

Additionally, results of parallel experiments, carried out with broth containing 1 % yeast sodium ribonucleate (cf. Table 10.) and with Bernheimer's basal medium containing Core II 0.6 mg/ml (cf. Table 11.), were also presented.

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

Part 21. A Simplified Method for Isolating High Potency Streptolysin-S Preparation

Akira FUJIMURA

Department of Pharmacology, School of Medicine, Kanazawa University
(*Director: Prof. Hajime OKAMOTO*)

Received for publication, July 22, 1963.

A very simplified method was described, whereby a high potency streptolysin-S preparation (minimum hemolytic concentration=1:100~200 millions) from culture fluid of *Streptococcus hemolyticus* grown on 1% commercial yeast nucleic-acid broth containing 1 mg RNase I per 100 ml broth.

The main advantage of the present method over the method of isolation of high potency streptolysin-S preparation from culture fluid of hemolytic streptococci grown on 1% Core II (or I)-broth is that commercial yeast nucleic-acid can be directly, but together with a minute amount of pancreatic ribonuclease, used for the preparation of culture medium. It is therefore not necessary to separate Core (AF) from the ribonuclease digest of yeast ribonucleic acid before the preparation of the culture medium.