Abstracts

1. BASIC STUDIES IN INH-THERAPY

Part 1. INH Concentration in the Serum After the Single Oral Administration

Takeshi YOKOI

Department of Bacteriology and Immunology,
Research Institute of Tuberculosis,
Kanazawa University
(Director: Prof. Masamichi KAKISHITA)

Received for publication, Nov. 29, 1962

Since INH is well known to have the property of being inactivated by acetylation in the body, the author carried out estimation of the concentration of biologically active INH in the serum of human subjects receiving oral administration of the drug.

I. Measurement of the INH concentration in serum 4 hours after administration of 4 mg of the drug per kg body weight was carried out on 79 patients with pulmonary tuberculosis, and the following results were obtained.

1) Remarkable individual difference was observed in the INH concentration, the values ranging as widely as from 0.12 to 2.0 μgm per ml.

2) The patients were divided into the following three groups.

Group 1: rapid inactivators showing concentrations lower than 0.25 μgm per ml.

Group 2: intermediate inactivators showing concentrations between 0.25 and 1.2 μgm per ml.

Group 3: slow inactivators showing concentrations higher than 1.2 μgm per ml.

Group 1 accounted for 31.6% of the patients, group 2 for 62.2%, and group 3 for 6.3%.

3) The majority of female patients were rapid inactivators, and the majority of male patients, intermediate inactivators.

4) No correlation was found to exist between the B.S.P. titer and the INH concentration.

II. Measurement of the INH concentration in serum after oral administration of 300 mg of INH per person was carried out on 106 patients with pulmonary tuberculosis and 22 healthy subjects, and the following results were obtained.

1) The serum INH concentration 4 hours after the administration lay betwe en 0.12 and 4.0 μgm per ml in the patients and between 0.12 and 2.0μgm per ml in healthy subjects, being conspicuously higher in the latter.

2) No correlation was found to exist between the body weight and the INH concentration.

3) Comparison of the concentrations at the fourth hour and the sixth showed the decrease to be greater in the slow inactivators than in the rapid inactivators.

4) The INH concentrations in serum and urine were estimated periodically in 10 of the patients, and it was found i) that the concentration reached the maximum two hours after the administration in most of them, and ii) that the concentration in urine rose and fell in parallel with that in serum, but was always much higher than the latter.

2. ANTIBODY-FORMING ABILITY OF RABBITS INJECTED NEONATALLY WITH BUFFALO BLOOD ALBUMIN

KEN-ICHI KONISHI AND MASAHIRO YOSHIMURA

Departments of Immuno-pathology and Pharmacology,
Research Institute of Tuberculosis, Kanazawa University
Received for publication, Nov. 30, 1962.

Rabbits intraperitoneally injected daily with 2 mg of buffalo blood albumin (BBA) 21 times from birth were divided into 5 groups. Three of the groups were challenged weekly with 40 mg of BBA for 3 weeks from 96, 175 and 188 days after birth respectively, and the other 2 groups weekly with 40 mg of human serum gamma globulin (HGG) for 3 weeks from 96 and 188 days after birth respectively. Control adults rabbits, which received no neonatal injection of BGG, were also treated in the same way with BBA or HGG. The antibodies responsible for BBA and HGG were examined by precipitation and complement fixation tests.

No antibodies for BBA could be detected in the rabbits of any of the 3 groups treated neonatally with BBA and challenged 3 times with the same antigen, while antibodies for HGG were clearly demonstrated in the rabbits of both groups treated neonatally with BBA, as well as in the controls, when they were challenged with HGG.

From these experiments it has been concluded :

1) Acquired immnunological tolerance is induced in rabbits receiving daily injection of 2mg of BBA for 21 days from birth.

2) The acquired immunological tolerance is retained at least till 188 days after birth.

3) The formation of antibody to HGG is not inhibited by the neonatal injection of BBA.

4) The phenomenon of immunological tolerance is specific to each antigen.

3. A CASE OF INTRATHORACIC NEURINOMA

NAOMASA MURAKAMI*, TOHEI KUROTA** AND HAJIMU ASAJI**

** Department of Clinical Research, Institute of Tuberculosis, Kanazawa University*
(Director: Prof. Miyoshi URABE)

***Toyama City Hospital, Toyama*

Received for publication: Nov. 10, 1962.

A case of sucessful operation of neurinoma of right posterior intrathoracic wall is reported. The patient was a woman of 21 and the tumor was discovered accidentally by chest X-ray examination when she was asymptomatic. Several months afterward she had neuralgia on the right chest. On opration the tumor was found to have developed on the 6th intercostal nerve and to be histologically a neurinoma. The postoperative course was uneventful and there was recurrence.

4. INFLUENCE OF THE DYES OF TRYPAN BLUE SERIES ON THE BACTERIAL HEMOLYSINS, TYROTHRICIN, GRAMICIDIN AND TYROCIDINE

MARIKO AKIYAMA, TOSHIHIRO NANBU AND OTO-O MIYAMOTO

Department of Pharmacology, Research Institute of Tuberculosis, Kanazawa University

(Director: Prof. Ryo ITO)

Received for publication, Oct. 15, 1962.

Experiments were made to ascertain whether dyestuffs of the trypan blue series, previously shown to exert an exceedingly strong inhibitory effect upon the hemolytic action of streptolysin S, produced similar inhibition against tyrothricin, gramicidin and tyrocidine, potent hemolysins isolated from cultures of Bac. brevis.

The dyes used were trypan blue, trypan red, congo red and thiazin red. The hemolysins were dissolved in alcohol (stock solution). For hemolysis tests the alcholic stock solution of the lysins were serially diluted with phosphate-buffered saline containing a dilution of the dyes. To 1 ml of these serial dilutions was added 1 ml of a 1% suspension of rabbit's erythrocytes, and then the mixtures were incubated at 37°C for 2 hours. In the controls the hemolysins were diluted with saline

containing no dyes. At the end of the incubation period the hemolytic titrations of both experiments, test and control, were read.

The results of these comparative hemolysis experiments revealed that none of the dyes tested exerted any demonstrable inhibitory effect on the hemolytic action of tyrothricin, gramicidin or tyrocidine.

5. STUDIES ON THE TYROSINE IODINASE

— ON THE ANTIGEN COMPONENTS OF ITS APOPROTEIN AND ON THE NATURE OF ITS COFACTOR —

MAMORU IWAKURA

Pathological Department, School of Medicine, Kanazawa University
(Director: Prof. Tachio ISHIKAWA)
Department of Bacteriology and Immunology, Research Institute of Tuberculosis, Kanazawa University
(Director: Prof. Masamichi KAKISHITA)

Received for publication, Nov. 30, 1962.

In a previous study it was observed that the enzyme which catalyses the biosynthesis of monoiodotyrosine (MIT) can be divided into two fractions. The one is a protein purified from the bovine submaxillary tissues and the other is a dialyseable anionic substance purified from the bovine thyroid gland. Furthermore, it was demonstrated that addition of cupric ion was not essential for this catalytic reaction but it increased the reaction velocity.

In this paper, in order to clarify the mechanism of the biosynthesis of MIT, apporotein of tyrosine iodinase were analysed immunochemically on its antigenic constituents and the nature of cofactor were also pursued.

The result obtained were summarized as follows;

1. By mean of agar gel double diffusion technique, the apoprotein purified by rechromatography on DEAE cellulose column were showed to be a homogeneous antigen.

2. It was found that the apoprotein obtained from thyroid gland had some antigens in common to apoprotein from submaxillary tissues, but a few non-identical precipitin lines were also observed by Ouchterlony method. Both apoproteins had immunologically no relationship with thyroglobulin.

3. The addition of fructose, glucosamine, ascorbic acid or sialic acid (NANA and N. O-DANA) in place of cofactor to the reaction mixtures were resulted formation of MIT respectively, without supplementation of cupric ion.

4. The cofactor seems to be identical with sialic acid by the both determination of colorimetric measuring and its chemical properties.

6. METABOLISM OF L-RHAMNOSE IN ESCHERICHIA COLI

HIDEO SAWADA

Department of Biochemistry, School of Medicine, Kanazawa University

(Director: Prof. Yasuyuki TAKAGI)

Received for publication, October 22, 1962.

The metabolism of methylpentose is receiving much attention because of its wide distribution in various biologically active substances. It has been described that this compound is converted to 3-carbon fragments in several kinds of microorganism. But the precise fate of the compound has not been revealed. Therefore the enzymatic cleavage of L-rhamnose by *Escherichia coli* was studied. The enzymes catalyzing the series of reactions involved in the metabolism of L-rhamnose were isolated from extracts of cells grown on the compound. The subjects of the present communication are the procedure for the purification of those enzymes and their properties.

1. The first step in the metabolism of L-rhamnose is isomerization to L-rhamnulose. A rhamnose isomerase has been purified about 50 fold from the crude extracts. It is an SH enzyme, specific for L-rhamnose, and no other sugar has been found to react with it. Only manganous ion is effective for activity. This enzyme catalyzes the reversible reaction, and at equilibrium about 60 percent of the total methylpentose is present as L-rhamnulose.

2. The second step is esterification of L-rhamnulose to yield a phosphate ester. An enzyme responsible for this reaction, L-rhamnulokinase, has been purified about 13 fold.

It is also an SH enzyme, and analytical studies indicate that the enzymatic product is L-rhamnulose 1-phosphate.

3. The crude extracts contain an enzyme which decomposes the phosphate ester quantitatively into an equimolar mixture of dihydroxyaceton phosphate and L-lactaldehyde. This enzyme, for which the name L-rhamnulose phosphate aldolase is proposed, has been purified about 15 fold. The enzyme catalyzes the reversible reaction. The phosphate ester of L-rhamnulose was found in the reaction mixture containing dihydroxyaceton phosphate and L-lactaldehyde added as the substrates, while 6-deoxy D-sorbose was found when the L-lactaldehyde was replaced with the D-isomer.

Publications not appealing in the Ann. Rep. Tbc. Kanazawa (1962)

Koshiura, R., Kagotani, Y. and Ujiie, T.: Experimental Anticancer Studies. XVI. Preparation and Anticancer Activity of 4-Amino-6-hexylresorcinol on Ehrlich Carcinoma in Mice. Chemical & Pharmaceutical Bulletin, **10** (6), 525, 1962.

Two compounds of Schiff's base type, in the chemical constitution of which the R−N=N−R' of 4-hexyl-6-(2-hydroxy-3, 5-dibromophenylazo)-resorcinol molecule is replaced by either R−CH=N−R' (No.191-A) or R−N=CH−R' (No. 191-B), and their original materials, [4,6-dibromo-2-aminophenol (No. 195), 4-amino-6-hexylresorcinol (No. 196), 2-hydroxy-3,5-dibromobenzaldehyde (No. 197) and 2,4-dihydroxy-5-hexylbenzaldehyde (No. 198)], were tested for their antitumor effect on Ehrlich carcinoma in mice. All the compounds were given in a daily dose of 1/5 LD_{50} into the peritoneal cavity of the experimental animals for 7 successive days, being initiated the first treatment 24 hours after implantation of 3×10^6 carcinoma cells.

1) Experiments, in which the experimental animal was implanted intraperitoneally with carcinoma cells: It was found that both No. 196 (LD_{50} 50mg/kg) and No. 191-A (LD_{50} 300mg/Kg) were very effective in prolongating the life-span of the experimental animals. But, No. 191-B, No. 195, No. 197 and No. 198 were all tested to be ineffective. On the other hand, comparative experiment carried out under similar conditions have shown that No.196, No.191-A and Mitomycin were most effective, followed by 2,6-bis-(2-hydroxy-3,5-dibromophenylazo)-4-propylphloro-glucinol (AZO-106), 6-mercaptopurine and N,N',N''-triethylenethio-phosphoramide (TESPA) in that order.

2) Experiments, in which the experimental animal was implanted subctaneously with carcinoma cells: In this kind of experiments, No. 196 and No. 191-A were tested to be less effective in inhibiting the growth of subcutaneous tumor in mice than 6-mercaptopurine. Comparative experiment with other anticancer agents have shown that 6-mercaptopurine was most effective, and No. 191-A the least effective, and mitomycin, TESPA, No. 196 and AZO-106 laid between the two in such order: 6-mercaptopurine>Mitomycin>TESPA≒No. 196>AZO-106≒No. 191-A.

Takano, T.: Surgical Treatment of Pleural Fluid. J. Chest. Dis., **6**(6), 766, 1962. Most important and practical problem of the srugical treatment against pleural fluid was reported with operative cases in our clinical labolatry and a review of the literature was discussed.