

# ABSTRACTS

## 1. BASIC STUDIES IN INH THERAPY

### PART 2. BLOOD INH LEVEL IN COMBINED ADMINISTRATION

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#### Experiment I.

The experiment was carried out to learn how the blood INH level estimated some hours after administration of the drug was affected by simultaneous administration of other drugs.

First, 300 mg of INH by itself was given orally to 116 patients with pulmonary tuberculosis, and the blood INH level was estimated 4 hours and 6 hours after that. The procedure was repeated several days later on the same patients, some other drug, however, being added to the INH given to each of them. The results are tabulated in the paper and may be summarized as follows.

1) The blood INH level was slightly raised by PAS-Ca and by PAS-Na-glucoside, but hardly at all by PAS-CON.

2) Among sulfamines, the largest effect of raising the INH level was shown by sulfamethomidine, which was followed by sulfaphenazole, sulfisomidine, and sulfisoxazole in that order. The level was lowered, by sulfamethoxypridazine and sulfaisomezole.

3) Among liver tonics and cholagoges, dehydrocholic acid had a strong effect of raising the INH level, methionine a less strong effect, and ursodeoxycholic acid only a weak effect.

4) Among all the substances tested, glucose had the strongest effect of lowering the level, and was followed by Rovin (an antidiabetic) and glucosamine-HCl in that order.

5) Of the two albumin-anabolic hormones, Durabolin lowered the level, and Abirol raised it.

6) Two furan derivatives, Puradin and Milanon, and an adrenocortical hormone Predonine were found to raise the level.

7) Vitamin B<sub>2</sub> in adequate doses markedly raised the level, while vitamin B<sub>1</sub> and calcium pantothenate were without effect.

8) No change of the level was caused by antihistamine or potassium iodide.

## Experiment II.

INH (300mg each) was given to 12 patients with pulmonary tuberculosis, and the blood INH levels were estimated 4 hours and 6 hours later. Several days afterward INH was again given to the same patients, this time 600mg each, and estimation of the levels was carried out as before.

The level was always higher after the second administration, and the increase was the more conspicuous the lower the initial level had been.

## Summary of the experiments

drug combined with INH	dose	the mean serum INH concentration after administration of INH alone		relative concentration after combined administration		no. of cases
		4 hrs.	6 hrs.	4 hrs.	6 hrs.	
antituberculous drugs						
PAS						
PAS-cal	2.0gm	0.93	0.46	1.11	1.15	42
PAS-Na-glucoside	2.0gm	1.00	0.50	1.10	1.20	5
PAS-CON	200mg	1.50	0.75	1.07	0.99	9
sulfamin						
sulfisoxazole (Thiasin)	1.0gm	0.88	0.51	1.15	1.00	28
sulfisomidine (Domian)	1.0gm	0.90	0.50	1.22	1.00	5
sulfamethoxy-pyridazine (Lederkyn)	0.5gm	0.97	0.43	0.82	0.88	5
sulfisomezole (Sinomin)	1.0gm	0.90	0.30	0.83	1.00	5
sulfmethylthiadiazole (Urocydal)	1.0gm	1.00	0.70	1.10	0.71	5
sulfaphenazole (Merian)	1.0gm	2.75	1.31	1.23	1.67	4
sulfamethomidine (Methofadin)	1.0gm	0.45	0.23	1.78	1.56	5
sulfadimethoxin (Abcid)	1.0gm	0.90	0.35	1.00	1.14	5
pyrazinamide (PZA)	1.0gm	1.00	0.50	1.10	1.00	5
liver tonic-cholagogics drug						
ursodesoxycholic acid (Urso)	25mg	0.95	0.50	1.32	1.28	14
	50mg	1.17	0.53	1.31	1.26	9
dehydrocholic acid (Dehychol)	500mg	0.94	0.47	1.50	1.64	8
methionin	0.5gm	0.89	0.50	1.31	1.12	9
glucosamine-HCl	300mg	0.75	0.35	0.73	0.80	5
	1.0gm	1.80	0.85	0.89	0.82	5

vitamins						
vitamin B <sub>1</sub>	5mg	0.90	0.50	1.11	1.00	5
	10mg	0.78	0.36	0.90	1.06	10
vitamin B <sub>2</sub>	2.1mg	0.78	0.56	0.92	0.77	9
	6mg	1.00	0.70	1.70	1.29	5
calcium pantothenate (Pancal-G)	1.0gm	0.89	0.53	1.00	0.89	9
antihistamine (Plokon)	0.5gm	0.81	0.50	0.93	0.68	8
glucose	0.5gm	1.08	0.69	0.54	0.64	10
albumin anabolic hormones						
Durabolin	25mg	0.50	0.25	0.76	1.00	2
Abirol	10mg	1.30	0.70	1.08	1.36	5
furan derivative						
furazolidons (Puradin)	100mg	1.39	0.94	1.35	1.80	4
Milanon	200mg	1.20	0.50	0.83	1.60	5
antidiabetic drug (Rovan)	0.5gm	1.33	0.83	0.75	0.51	3
adrenocortical hormone (Predonine)	5mg	1.42	0.57	0.90	1.42	9
potassium iodide	50mg	1.21	0.66	1.07	1.12	14

## 2. BASIC STUDIES IN INH THERAPY

### PART 3. CORRELATION BETWEEN THE BLOOD INH LEVEL

#### AND THE THERAPEUTIC EFFECT

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Observations were made on 101 patients with pulmonary tuberculosis treated with INH for 3 to 24 months to find the correlation between the therapeutic effect of the drug and the blood INH level. The results obtained were as follows :

1) The erythrocyte sedimentation rate is evidently related to the blood INH level. Marked reduction of the rate was observed in the patients whose blood INH levels were 0.5  $\mu$ gm per ml or higher (high-level group).

2) No correlation was observed to exist between the increase of body weight and the INH level.

3) Disappearance of tubercle bacilli from the sputum was observed in a larger percentage of patients of the high-level group than the other group, both by the smear-stain method and by the culture method.

4) The chest X-ray picture showed disappearance of cavities to be commoner in the high-level group than in the other.

5) Appearance of INH resistant bacilli was also more frequent in the high-level group.

### 3. IMMUNOLOGICAL STUDIES ON SENSITIZED ERYTHROCYTES

#### PART 19. IMMUNOLOGICAL SIGNIFICANCE OF ERYTHROCYTES SENSITIZED WITH THE HEAT-EXTRACT OF SHIGELLA

##### No. 1. UPON THE ANTIGENICITY OF THE HEAT-EXTRACT OF SHIGELLA

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In order to investigate the antigenicity of heat-extract (E) from Shigella, the following two groups of rabbits, each consisting of three subgroups, were employed in this experiment.

Group A: Rabbits injected with Shigella vaccine

Subgroups A<sub>1</sub>, A<sub>2</sub> and A<sub>3</sub>: Rabbits injected with Sh. flexn. 2a(2a),  
Sh. flexn. 1b(1b) and Sh. sonnei (son.) vaccine, respectively

Group B: Rabbits injected with red cells sensitized with E

Subgroups B<sub>1</sub>, B<sub>2</sub> and B<sub>3</sub>: Rabbits injected with red cells sensitized with  
E from 2a, 1b and son., respectively

On each of the sera prepared from these animals agglutination, precipitation and hemolysis reaction tests were carried out with homologous and heterologous vaccines, E's and E-sensitized red cells.

The results obtained were as follows:

1) Rabbit red cells were easily sensitized with E's, and antibodies were produced in rabbits injected with their own red cells sensitized with E. (Group B)

2) However, both agglutinin and precipitin titers were lower in group B than in group A.

3) The sera of rabbits of subgroups A<sub>1</sub>, A<sub>2</sub> and A<sub>3</sub> were observed to react more specifically with E-sensitized red cells from 2a, 1b and son. than with 2a, 1b and son.-vaccine, respectively. In particular no hemolysis of red cells sensitized with E from 2a or 1b was caused by the antisera of rabbits of subgroup A<sub>3</sub>, and conversely no hemolysis of red cells sensitized with E from son. by the antisera of rabbits of subgroup A<sub>1</sub> or A<sub>2</sub>.

4) E's from 2a, 1b and son. were observed to react specifically with the sera of rabbits of subgroups A<sub>1</sub>, A<sub>2</sub> and A<sub>3</sub> respectively in precipitation reaction test and hemagglutination and hemolysis inhibition reaction tests.

5) Absorption tests, revealed that antibodies not found in the sera of rabbits of subgroups of B<sub>1</sub>, B<sub>2</sub> and B<sub>3</sub>, existed respectively in the sera of rabbits of subgroups A<sub>1</sub>, A<sub>2</sub> and A<sub>3</sub>.

#### 4. IMMUNOLOGICAL STUDIES ON SENSITIZED ERYTHROCYTES

##### PART 19. IMMUNOLOGICAL SIGNIFICANCE OF ERYTHROCYTES SENSITIZED WITH THE HEAT-EXTRACT OF SHIGELLA

##### NO. 2. UPON THE ANTIGENICITY OF THE FRACTIONS FROM THE HEAT-EXTRACT OF SHIGELLA

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For the purpose of clarifying the nature of the red-cell-sensitizing factor of heat-extracts (E's) of *Shigella*, the author prepared polysaccharide and protein fractions (CF and PF, respectively) from the E's of S. 2a, S. 1b, and S. sonnei and tested their erythrocyte-sensitizing activities by means of the six antisera prepared by the method reported in the preceding paper (Part 19, No. 1). The results obtained were as follows:

1) The CF and PF from the three strains were all capable of sensitizing rabbit red cells.

2) These fractions, as well as red cells sensitized with them, were found to

react specifically with the respective homologous anti-sera.

3) No hemolysis of red cells sensitized with the CF from 2a or 1b was caused by any anti-sonnei serum, either anti-vaccine or anti-sensitized-cell. Conversely no hemolysis of red cells sensitized with the CF from sonnei was caused by any anti-2a or anti-1b serum of either kind.

4) The pattern in Ouchterlony's double diffusion test plate indicated that each of the three anti-vaccine sera contained at least three kinds of antibodies, i. e. one which reacted only with E, another which reacted with E and CF, and the third which reacted with all the three, E, CF, and PF.

5) The absorption tests revealed that the antigenic difference between the CF and PF was less pronounced in the case of sonnei than in 2a or 1b.

## 5. STUDIES ON THE RESISTANCE OF MICROORGANISMS TO VARIOUS CHEMICALS

### PART 19. STUDIES ON THE RESISTANCE OF COLIBACILLUS

#### AGAINST VARIOUS ANTIBIOTICS

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Experimental studies on the resistance of Colibacillus against SM, CM, TC and KM were carried out, and the following results were obtained :

1) The colibacillus in the feces of the subjects receiving SM, CM or TC alone was observed to be resistant not only to the particular drug but also to the other two. It was, however, not resistant to KM.

2) The feces of the subjects receiving KM, either in 100 daily subcutaneous injections of 1gm each or in 10 daily oral doses of 1gm each, were found to be free from colibacillus for about seven days after the termination of medication, and the bacilli that reappeared thereafter were sensitive to KM.

## 6. EXPERIMENTAL ANTICANCER STUDIES

### PART 18. PREPARATION AND ANTICANCER ACTIVITY OF 6-ALKYL

#### DERIVATIVES OF 4-AMINORESORCINOL

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Nine 4-amino-6-alkylresorcinols, i.e. ethyl-, n-propyl-, n-butyl-, n-amyl-, iso-amyl-, n-hexyl-, iso-hexyl-, n-octyl- and n-decyl-derivative, were synthesized. Among them, 4-amino-6-n-octylresorcinol was found to be most effective in inhibiting the growth of both the ascites and solid forms of Ehrlich carcinoma in mice.

## 7. STUDIES ON THE COFACTOR NECESSARY FOR ACTIVITY OF RIBONUCLEIC ACID IN INDUCING STREPTOLYSIN S

### PART 3. COFACTOR ACTIVITY TEST WITH AMINO ACIDS, PEPTIDES, CARBOHYDRATES AND NUCLEIC-ACID COMPONENTS

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It was shown firstly by Bernheimer and Hosoya et al. (1948-49) that a cofactor (or cofactors) for streptolysin S inducing effect of RNA was presented in both meat infusion and peptone.

Recently, Shimizu has succeeded in isolating a non-dialysable peptidic fraction with potent cofactor activity from meat infusion, and Shoin a highly active peptidic fraction from dialysable portion of commercial peptones.

On the other hand, there is a Bernheimer and Rodbart's work, in which it was demonstrated that both maltose and glucosamine, in a minute amount, could effectively be substituted by peptone or meat extract in the streptolysin S inducing experiment with RNase core.

In view of these informations and results obtained in our anticancer experiments with hemolytic streptococci, it was of interest to extend the study of cofactor. In the present study, 67 organic substances, including amino acids, synthetic peptides, carbohydrates, nucleic-acid components etc., were tested for their cofactor activity, employing the method of resting cell system.

The principal results obtained are as follows :

1) None of 18 amino acids (glycine, D-alanine, L-alanine,  $\beta$ -alanine, L-leucine, L-isoleucine, L-lysine, L-glutamic acid, DL-valine, DL-tryptophane, DL-ornithine, L-tyrosine, L-phenylalanine, L-arginine, DL-threonine, L-histidine, DL-methionine and carnosine) was tested to be active. However, potent cofactor activity was expected with any of three commercial preparations of amino acid mixture (composed of L-lysine, L-threonine, L-methionine, L-tryptophane, L-leucine, L-isoleucine, L-phenylalanine, L-valine, L-arginine, L-histidine and glycine) now available in Japan.

2) The peptide fraction (FII $\alpha$ ), prepared according to the method described by Shimizu from meat infusion, was found to be the most potent cofactor among 67 substances. Seven synthetic amino acid polymers were proved to be not active at all.

3) Sucrose was tested to be as active as maltose, and di-glucose-6-phosphoric acid and uridine were less active.

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## 8. METHYLATED ALBUMIN CHROMATOGRAPHICAL PURIFICATION OF STREPTOLYSIN S SAMPLE

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1) Partially purified streptolysin S sample (I-N-F fraction) was first prepared according to Okamoto and Shoin from 30-hour culture of *Streptococcus hemolyticus* grown on meat-infusion broth containing 1 % yeast ribonucleate. The hemolytic activity of the fraction was 100 hemolytic units per optical density at 260 m $\mu$ .

2) Methylated albumin chromatography was successfully used for further purification of the I-N-F fraction. The 0.5 M NaCl eluate thus obtained showed 6,000 hemolytic units per optical density at  $260\text{ m}\mu$ ; The hemolytic power of the eluate was therefore 60 times as great as that of the starting material, I-N-F fraction.

3) Quite similar result was obtained in the chromatographical experiment performed with the I-N-F sample from the 1 % yeast RNase core-broth culture of the cocci.

## 9. STUDIES ON BIOSYNTHESIS OF MONOIODOTYROSINE

### I. PURIFICATION AND PROPERTIES OF THE ENZYME

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1. By the purification of the moniodotyrosine synthesizing enzyme can be separated into apo-protein and a cofactor.

2. The apo-protein has been purified from bovine submaxillary tissues by chromatography on hydroxylapatite column. Paper electrophoresis and ultracentrifugation are made to determine the homogeneity of the preparation.

3. The cofactor has been purified from bovine thyroid tissues by chromatography on Dowex I column.

4. It is discussed that this enzyme system does not contain peroxidase or xanthine oxidase which is believed to participate in oxidation of iodide.