Abstracts

1. THE TOXICITY OF SALINE EXTRACT OF TUBERCLE BACILLI FOR MICE (Part 1)

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The present paper is concerned with the observation that intravenous injection of saline extract of human tubercle bacillus, strain Aoyama B, caused toxic death of *dd* mice within a few minutes.

The bacilli were grown on Sauton's medium for about 2 weeks. The saline extract was prepared by grinding and then suspending 1 gm (semi-moist weight) of washed tubercle bacilli in 5 ml of saline, followed by centrifugation of the suspension at 10,000 g for 30 minutes. The extract thus obtained, when given intravenously in a dose of 0.3 to 0.5 ml, caused lethal toxicity on mice weighing about 15 gm, but, when given subcutaneously or intraperitoneally, showed no toxic effect. The mice which died from intravenous injection of the extract showed, in autopsy, considerable congestion of various visceral organs.

The factors influencing the toxicity of the extract were studied with the following results:

1) All the human and bovine strains of *M. tuberculosis* were demonstrated to give highly toxic saline extracts, while the extracts prepared from the avian, timothy and atypical tubercle bacilli were all found to be devoid of toxicity.

2) The toxicity of the extract was (a) nondialysable, (b) inactivated by heating at 100°C for 30 minutes and by treatment with HCl, acetic acid and NaOH, and (c) precipitated by methanol, ethanol and acetone.

2. THE TOXICITY OF SALINE EXTRACT OF TUBERCLE BACILLI FOR MICE

Part 2. Effect of Various Substances, Including Streptolysin S, Enzymes and Adrenaline, on the Toxicity of Saline Extract

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In the preceding report Yoshimura et al. reported the toxic action on mice of the saline extract of human as well as bovine tubercle bacilli. The present paper is concerned with the effect of various substances on the toxicity of the extracts.

The saline extract of tubercle bacilli prepared from human tubercle bacillus, strain Aoyama B, according to the method described previously was injected into the tail vein of *dd* mice. The substances tested in this study were (1) a purified sample of streptolysin S and sodium ribonucleate; (2) proteinases (pepsin, trypsin, papain and erepsin), lipase, α- and β-amylase and β-glucosidase; and (3) adrenaline, ephedrine, and four antihistaminics (diphenhydramine, promethazine, dimenhydrinate and chlorhetramine).

The results obtained may be summarized as follows:

1) When streptolysin S was mixed with the saline extract of tubercle bacilli before the injection, the mice survived the injection, as little as 0.01 mg of the substance being enough to inhibit the toxic effect of the extract. Inactivation of the lysin by heat hardly affected its inhibitory action. Sodium ribonucleate showed same inhibitory action on the extract, but it was hundreds of times less effective than streptolysin S.

2) The toxic activity of saline extract was readily destroyed by pepsin as well as lipase, while none of the other enzymes studied showed such effect.

3) Intraperitoneal pretreatment with adrenaline (0.05 mg) or ephedrine (0.5 mg) protected the mice from intoxication by subsequent injection of a toxic dose of the saline extract, whereas similar pretreatment with the antihistaminics produced no protective effect.

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

Part 2. Effect of Various Substances on Tanned-Erythrocyte Agglutination, with Special Reference to the Inhibitory Effect of Serum Glycoprotein

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In the previous report was described a new type of hemagglutination between tannic acid-treated erythrocytes and the serum of guinea pigs. The present paper is concerned with the effect of a large variety of substances on the tanned erythrocyte (TE) agglutination of tuberculous guinea-pig serum.

The substances tested included proteins, amino acids, carbohydrates, nucleic acids and nucleotides, chelating agents and enzyme poisons. Guinea-pig erythrocytes were thoroughly washed in phosphate-buffered saline (pH 7.2) and treated with a 1 : 10,000 solution of tannic acid in a cold room (2° ± 2°C) for 30 minutes, then washed in cold saline and finally resuspended in saline to the original blood volume. The serum was obtained from tuberculous guinea pigs infected, at least one month before, with human tubercle bacillus, H37Rv.

To 2 ml of each serial dilution of a substance to be tested was added 2 ml of an appropriate dilution (1 :100-250) of tuberculous serum and 0.2 ml of tanned erythrocyte suspension. In a control tube, the solution of the substance was replaced with saline. The mixture was allowed to stand at room temperature for 25 minutes. Readings were taken at 5-minute intervals.

Among the 115 substances tested, the glycoproteins from human as well as bovine serum were found to be the most potent inhibitor to the TE agglutination of tuberculous guinea-pig serum. A concentration as low as 1 : 640,000 of these glycoproteins was effective to exert a recognisable inhibition. Much lower degrees of inhibition were observed in β-lipoprotein, heparin, EDTA, chondroitin sulfate, gallic acid, gentisic acid, protocatechuic acid, salicylic acid, pyrogallol, 8-hydroxyquinoline, pyridoxal phosphate, anthranilic acid, RNA, DNA, guanylic acid, monoiodoacetic acid, malonic acid, 2,4-dinitrophenol and kanamycin.

4. DEVELOPMENT OF TUBERCULIN SKIN HYPERSENSITIVITY AND FORMATION OF CIRCULATING ANTIBODY IN RABBITS INJECTED NEONATALLY WITH HEAT-KILLED BCG

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Rabbits receiving daily intraperitoneal injection of 0.1 mg of heat-killed BCG for 21 days following the birth were challenged intradermally with 1.0 mg or 0.1 mg of living BCG on the 155th day after birth. Control rabbits, about 140 days old, which received no neonatal injection of killed BCG, were also challenged in the same way. After the challenge the antibody production was examined by tuberculin skin test, hemagglutination and hemolysis tests.

The results showed that in the rabbits treated neonatally with killed BCG the development of tuberculin skin hypersensitivity was more or less inhibited and the production of circulating antibody was suppressed markedly though not completely.

5. TUBERCULIN SKIN HYPERSENSITIVITY AND RESISTANCE AGAINST TUBERCULOUS INFECTION OF GUINEA PIGS TREATED NEONATALLY WITH OLD TUBERCULIN "BCG"

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Three groups of rabbits were subjected to experiments carried out to investigate the influence of neonatal injection of OT on the development of OT skin reactivity and of resistance against tuberculosis.

Group A : nine guinea pigs injected neonatally with OT-BCG by intraperitoneal route and inoculated intradermally with 0.1 mg of living BCG 3 to 4 months after birth.

Group B : six guinea pigs inoculated with living BCG as group A without previous injection of OT-BCG.

All the animals of groups A and B were tested for their OT skin sensitivity 28 days after the BCG inoculation and 3 days later infected subcutaneously with 0.1 mg of human type tubercle bacilli, $H_{37}Rv$ strain.

Group C : three control adult guinea pigs infected with $H_{37}Rv$ strain as groups A and B without any treatment.

All the three groups of animals were killed 5 weeks after the infection of $H_{37}Rv$ strain, and the lesions of the visceral organs were observed macroscopically, and quantitative culture of the bacilli in the visceral organs was performed.

The results obtained revealed that the neonatal injection of OT (1) suppressed the development of OT skin hypersensitivity due to BCG-inoculation, (2) but did not influence the protective effect of BCG against tuberculosis.

6. TUBERCULIN SKIN HYPERSENSITIVITY AND RESISTANCE AGAINST TUBERCULOUS INFECTION OF GUINEA PIGS INOCULATED WITH BCG AT BIRTH

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Twenty-one guinea pigs inoculated intraperitoneally with varying doses of living BCG within twelve hours after birth were tested for their tuberculin skin reactivity one and two months after the inoculation. One month after the second skin test all the animals were infected subcutaneously with 0.02 mg of human type tubercle bacilli, H_2 strain. Eight control guinea pigs not injected with BCG were also infected in the same way. The animals were sacrificed three months later, and the lesions of the visceral organs were observed and then quantitative culture of the bacilli present in the organs was performed.

The results obtained were as follows.

1) None of the animals injected with BCG at birth failed to show skin hypersensitivity to OT within one month after birth and to retain it at least for another month.

2) All the guinea pigs inoculated neonatally with BCG were observed to show high resistance to tuberculous infection.

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

Part 2. Experiment on the Prolongation of the Duration of Immunological Tolerance

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In the preceding paper the authors reported the acquired immunological tolerance for buffalo blood albumin (BBA) induced in rabbits by 21 daily injections of BBA from birth was retained at least till 188 days after birth. In this paper the results are described of the experiments carried out to determine how much longer the tolerance was retained.

Thirteen rabbits, intraperitoneally injected daily with 2 mg of BBA 21 times from birth, were divided into 4 groups ; Group A (2 rabbits) : challenged with BBA 96, 288, 411, 590 and 715 days after birth. Group B (2 rabbits) : challenged with BBA 175 (or 188), 334 and 455 days after birth. Group C (7 rabbits) challenged with BBA when 334 to 411 days old. (Four of them received human serum gamma globulin (HGG) between the 96th and 288th day after birth.) Group D (2 rabbits) : challenged with BBA when 538 days old.

Twelve control adult rabbits, which received no BBA, were also challenged with BBA or HGG 100 to 160 days after birth, and 4 of them were rechallenged 192 days later. The antibodies responsible for BBA and HGG were examined by precipitation and complement fixation tests. The results obtained were as follows.

1) The rabbits of group A were observed to be tolerant to BBA when 411 days old. Moreover, one of them was still tolerant on the 590th day after birth, but the other was not.

2) The rabbits of group B, challenged with BBA on the 175th or 188th day after birth and proved to be tolerant, were observed to be still tolerant when 334 days old. They had lost the tolerance completely by the time they were 455 days old.

3) Of the seven animals of group C challenged with BBA between the 334th and 411st days, three produced antibody of low titre, while the antibody titres of the other four were as high as those of the controls.

4) The rabbits reciving no additional treatment except neonatal injection of BBA produced anti-BBA when injected with BBA 538 days after birth.

These observations suggest that challenge injections given at certain intervals of time following the tolerance injection would work for prolongation of the tolerance.

8. BIOSYNTHESIS OF DEOXYRIBONUCLEIC ACID IN BACTERIOPHAGE T4-INFECTED ESCHERICHIA COLI K12(λ)

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The phage-induced initial reactions and the regulatory mechanism of early enzyme synthesis participating in the production of viral DNA were analyzed in *E. coli* K12(λ) after single or mixed infection with r^+ (wild type), H88 (a deletiontype mutant of the A-cistron class) and r196 (a deletiontype mutant of the B-cistron class) of T4 phage.

1) The synthesis of viral DNA proceeded vigorously after a lag period in mixed infection with H88 and r196 just as in r^+ infection. The DNA synthesis in cells infected separately with either of the rII mutants proceeded only during the early stage and the amount of DNA reached in 30 minutes 4～16% of that synthesized in r^+-infected cells.

2) The materials having absorption at 260mμ and the deoxyribonucleosidic compounds in the acid-soluble fraction of rII mutant-infected cells accumulated normally during the first 15 minutes and then remained at a constant value.

3) dCTPase and dCMP hydroxymethylase were induced in the host cell after infection with H88 or r196 as well as with r^+. The level of these enzymes reached a maximum in aproximately 10 to 15 minutes and then remained constant in both cases of infection with r^+ and with rII mutant.

4) When bacteria were infected with phage treated with ultraviolet light (UV) a striking divergence was observed between the behaviors of r^+ and rII mutants ; dCTPase synthesis by UV-r^+ and by simultaneous infection with UV-

H88 and UV-r196 continued beyond 15 minutes, but in contrast, the synthesis in the cell infected with UV-rII stopped after 15 minutes just as in the cell infected with non-irradiated rII. Therefore, it was concluded that in K12 (λ) receiving abortive infection with rII mutant, the general loss of synthetic capacities took place abruptly about 15 minutes after infection, as evidenced by the stoppage of DNA synthesis and the accumulation of acid-soluble materials.

5) The mode of dCTPase synthesis after simultaneous infection with one UV-irradiated and another non-irradiated complementary rII mutant (e.g. UV-H88+r196, H88 + UV-r196, UV-rII + r^+) revealed extended production of the enzyme beyond 15 minutes; viz. the UV-irradiated phage chromosome behaved as if it were dominant over the non-irradiated chromosome with respect to the mechanism for control of the level of infection-induced dCTPase. On the basis of these findings the regulatory mechanism governing early enzyme synthesis is discussed in this paper.

9. SERUM POLYUNSATURATED FATTY ACIDS IN INFANT NUTRITION

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The levels of the serum P. U. R. A. of 23 breast-fed infants and 39 bottle-fed infants were determined by the alkaline isomerizing method, and statitstical investigation was made of the data.

1) The bottle-fed infants, both mature and premature, gave lower levels of total P. U. F. A., di- and tetraenoic acids and higher levels of trienoic acid than the breast-fed.

The serum levels of di- and tetraenoic acids were much lower in the premature infants than in the mature ones.

2) Giving the bottle-fed infants powdered milk whose linoleic acid was 4% of the prematue calorie raised their serum P. U. F. A. levels to those of the breast fed.

3) It is suggested that the pattern of the serum P. U. F. A. of the premature infants fed with milk whose linoleic acid was 0.5% of the total calorie of indica-

tes a latent state of Essential Fatty acid Deficiency.

4) The pattern of di-, tri- and tetraenoic acids in the serum seemed to be an indicator of the fat-metabolism in infants, and a biochemical standard by which attificial feeding may be evaluated in comparison with breast feeding.

10. THYMINE-REQUIRING MUTANT OF *SALMONELLA TYPHIMURIUM* LT-2

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When *Salmonella typhimurium* LT-2 was incubated in Gray and Tatum medium or glucose-salts synthetic medium supplemented with aminopterin (AM, an antagonistic inhibitor of folic acid) and thymine (T), many thymine-requiring mutants (Thy^-) appeared in the culture. These mutants require the presence of high concentrations, e.g. 10μg/ml, for satistactory growth, and easily die out in about 1 hour of incubation in a medium lacking T (thymineless death), as reported about *E. coli* 15 Thy^-.

The author carried out experiments to analyze the mechanism of action of AM on the appearance of Thy^-. Several lines of evidence supported that AM doesnot have any mutagenic action and the growth of Thy^- strains is faster than that of Thy^+ strains in the presence of AM and T in the medium. Thus the results suggested that Thy^- cells were derived from the mutants which appeared spontaneously in the Thy^+ population during its growth (spontaneous mutant) and became predominant by the inhibitory action of AM on the growth of Thy^+ strains (selection).

Sulfanilamide, another antagonistic inhibitor of folic acid, seemed to have no significant selecting activity on Thy^- mutants.

11. ANESTHETIC MANAGEMENT FOR AGED PATIENTS

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Statistical analysis was made of the anesthetic procedure of 203 cases over 60 year old out of 1438 patients treated during the period from 1960 to 1962. The functional disorders particularly caused by hypertensive arteriosclerosis were observed to increase sharply with advance of age in the geriatric patients before operation. In 33% of these patients, marked fall of blood pressure was recognized at the induction of anesthesia. The disorder of the hepato-renal function observes after anesthesia and surgery also increased with age.

The observations of the clinical cases indicated that attention was to be paid for the anesthesia of the aged in the following way. The premedication should be done in small doses, drugs low in sideeffect being chosen. Barbiturate might be used for the indidation, if necessary, but only in minimal doses. The anesthesia should be kept light through-out the entire operation, G-O-E or G-O-F being employed with a muscle relaxant. Accurate and rapid replacement of the shed blood should be carefully made during the operation to prevent postoperative circulatory failure and diminish postoperative morbidity. Tracheotomy is the procedure of choice, when evidence of cardiac failure is observed, or the patient is not capable of expectoration, after the anesthesia.

12. EXPERIMENTAL ANTICANCER STUDIES

Part 19. Anticancer Activity of 2-[(5-Nitro-2-furyl) vinylene] quinoline Carboxylic Acid-(4)

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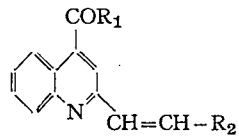
Sincs 1954, the investivators of our laboratory have been devoted themselves to the anticancer studies with a number of derivatives of 2,2'-dihydroxyazobenzene, Schiff base type and aminoalkylresorcinol.

In the course of the study, ten compounds of following type of chemical constitutions were also synthesized and tested for their anticancer activity.



Each of seven 2-substituted quinoline carboxylic acid-(4) derivatives was obtained by condensation of 2-methylquinoline carboxylic acid-(4) with the respective aromatic aldehyde in the presence of acetic anhydride or zinc chloride, according to the method described by Royer. Three hydrazides of 2-substituted quinoline carboxylic acid-(4) were prepared by acting hydrazine on each of 2-(styryl)-, 2-(*p*-aminostyryl)- and 2-(*p*-dimethylaminostyryl)-quinoline carboxylic acid-(4) esters. All these compounds were shown to be of ***trans*** configuration.

The results so far obtained in the anticancer experiments, in which each mouse was given intraperitoneally a daily dose of 1/5 LD_{50} of a test compound for seven successive days after inoculation of Ehrlich carcinoma cells, are as follows: Among ten compounds, 2-[(5-nitro-2-furyl) vinylene] quinoline carboxylic acid-(4) (No.256) was found to be most effective in causing prolongation of the life-span of mice bearing Ehrlich ascites carcinoma. It was also observed that the growth of solid form of the carcinoma was moderately inhibited by the compound.

Publications not appealing in the Ann. Rep. Tbc. Kanazawa (1964)

1) **YOSHIMURA, Masahiro and KABURAKI, Toshiko** : Immunization against Ehrlich mouse ascites carcinoma with chemically devitalized cells. Japan. J. Pharmacol., **13**, 127, 1963.

Ehrlich ascites tumor cells were exposed to the action of acetic anhydride, monoiodoacetic acid, Nitromin and nitrous acid. Tests for viability by transplantation of the treated tumor cells to *dd* mice showed that tumor cells lost their transplantability following exposure (a) 6% acetic anhydride at 0°C for 1 hour, (b) 0.1 M monoiodoacetate at 0°C for 1 hour, (c) Nitromin (25 mg/ml) at 37°C for 15 minutes and (d) 1 M nitrite at 0°C for 15 minutes.

Immunization experiments carried out with the lethally damaged tumor cells revealed (a) that a single intraperitoneal injection of the nitrite-inactivated cells caused an enhanced resistance against subsequent viable transplants, resulting in reduction of the mortality to about 50 per cent of the recipients; (b) that similar pretreatment either with Nitromin or acetic-anhydride inactivated cells caused some prolongation of survival times of mice; (c) and that, in contrast to these positive findings, no significant degree of protection was demonstrated following prior treatment of mice with the monoiodoacetate-inactivated cells.

2a) **ITO, Ryo and AKIYAMA, Mariko** : Hemagglutination phenomenon between tuberculous serum and tanned erythrocytes of guinea pigs. Japan. J. Tuberc., **10**, 102, 1962.

2b) **ITO, Ryo and AKIYAMA, Mariko** : A hemagglutination phenomenon between tanned erythrocytes and the serum of guinea pigs. Amer. Rev. Resp. Dis., **88**, 553, 1963.

A marked hemagglutination occurred when fresh serum from tuberculous guinea pigs acted upon a suspension of tannic acid-treated erythrocytes of normal guinea pigs. Such an agglutination could not be demonstrated either with heat-inactivated tuberculous serum or with normal serum. The demonstration of hemagglutination was influenced by various factors, the use of erythrocytes tanned at cold temperatures being the most important. Evidence was presented to indicate the possible presence of both agglutinator and inhibitor in the serum.