

ABSTRACTS

—Review—

SURGICAL TREATMENT OF CANCER

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A general review of surgical treatment of cancer is presented. The author does not think that we can expect conservative therapy such as chemotherapy to become so rational a way for curing cancer as chemotherapy with sulfonamide or antibiotics is for curing infectious diseases, because carcinostatic drugs only suppress the mitosis of cancer cells by acting as a cell toxin.

Needless to say, the surgical resection (or extirpation) of the cancerous tumor is at present the only dependable method of therapy, and it is important to detect cancer and to perform the curative radical operation as early as possible before it should become generalized. However, the so-called ultra-radical operation of cancer performed recently by some surgeons must be re-examined in the light of preservation of the protective power of the organism.

—Original—

1. STUDIES ON ATYPICAL ACID-FAST BACILLI

PART 2. ISOLATION OF ATYPICAL ACID-FAST BACILLI
FROM TUBERCULOUS PATIENTS

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Niacin tests of acid-fast bacilli from 574 patients with tuberculosis were carried out in our laboratory in 1962 and 1963. The bacilli from 4 patients showed negative reaction. The four strains were tested for a number of biological and biochemical characteristics, and three strains were found to be atypical acid-fast bacilli (one scotochromogen and two rapid growers), and the other strain to be the human tubercle bacillus.

2. METABOLISM OF LIPIDES IN THE TUBERCULOUS LESION

PART I. HISTOCHEMICAL STUDIES ON LIPIDES OF EXPERIMENTAL TUBERCULOUS RABBITS HIDEYUKI TAKATA, MASAHIRO SAITO, KENSUKE MURASAWA AND MIYOSHI URABE

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The authors have performed histochemical studies on lipides in the tuberculous lesions of the lung and liver of rabbits. The result obtained are as follows :

1. Fatty acid were found in tuberculous lesions of the lung and liver and their changes were paralell with the histological changes of the lesions. They increased in the period of intensive caseation and decreased in the process of healing. The fatty acids were mostly deposited outside of caseous lesions.

2. Differential determination of the fatty acids revealed the presence of magnesium salts of steric, palmitic and oleic acids. The magnesium and calcium salts chaged together in the pulmonary lesion, but in the hepatic lesion the calcium salts increased and magnesium salts decreased in the period of severe histological evidence.

3. Phospholipides appeared 1 week after the inoculation with tubercle bacilli and remained for a long time though the amount was very small. The fatty acids were deposited mostly outside of caseous lesions, and the phospholipide mostly inside. The phospholipide consisted mostly of recithin and sphingomyelin.

3. METABOLISM OF LIPIDES IN THE TUBERCULOUS LESION

PRAT II. BIOCHEMICAL ANALYSIS OF HIGHER FATTY ACIDS IN EXPERIMENTAL TUBERCULOUS LESIONS

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The change of higher fatty acids of the neutral fat was investigated by means of gas chromatography in the plasma and tuberculous lesions of the lung and liver, of the rabbit. The results obtained were as follows :

1. The higher fatty acids which composed the neutral fat of the plasma, lung and liver, were myristic, palmitic, palmitoleinic, stearic, oleic, linoleic and linolenic acids.

No particular fatty acid appeared in the tuberculous lesions.

2. The total neutral fat decreased when the histological evidence of tuberculosis became extensive. The higher saturated fatty acids increased, and the higher unsaturated fatty acids decreased in the plasma, lung and liver.

3. The pattern of changes of fatty acids following the inoculation of tubercle bacilli, was identical in the plasma, lung and liver, but temporal differences were recognized in the appearance of the peaks of change among the three.

4. The ratio of palmitoleic to palmitic acid fell in the plasma and pulmonary lesion 2 weeks after the inoculation, and the ratio of oleic to stearic acid decreased in the plasma, and pulmonary and hepatic lesions when the pathological changes became extensive, and the ratio of linoleic to oleic acid showed the highest value at the time of the most severe histological evidence of tuberculosis. The ratio of the unsaturated to the saturated fatty acid showed the least value 3 weeks after the inoculation.

4. EPIDEMIOLOGICAL OBSERVATIONS OF THE STATE OF TREATMENT OF TUBERCULOUS PATIENTS

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The authors conducted a survey of the registered patients in the area under the Kanazawa Central Public Health Office from 1959 through 1963, mainly about the acquisition of drug resistance by tubercle bacilli and the variation of the resistance, which pose a major problem in the treatment and control of tuberculosis.

1. The rate of shedding of drug-resistant bacilli rose steadily, and in 1963 it was found 54% of the specimens of bacilli set free by the patients had drug resistance. The patients shedding resistant bacilli were observed to have received chemotherapy in larger dosage and for longer periods than those shedding susceptible ones. The rate of restoration of susceptibility of the bacilli was lower in the group of patients receiving the three drugs simultaneously. But later field observations seemed to indicate that those patients were advanced cases.

2. Only 46 to 56% of the patients, that is considerably less than the national average, were found to be taking advantage of the government financing for treatment. Of those receiving treatment without government aid, 28% were receiving anti-tuberculosis drugs, and a large percentage were without occupation.

3. Perhaps owing to the revision of the law, the ratio of cases receiving treatment to the total cases of open tuberculosis began to rise in 1962. More and more patients resorted to the provisions of the Livelihood Protection Law and the national health insurance system. No patient in families covered by the Livelihood Protection Law has been found to go untreated.

4. A census-taking made in the area revealed no more than 70% of the patients being treated for tuberculosis were duly registered with the office as required by law.

5. MASS EXAMINATION FOR ADULT DISEASES PART 2

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Mass examination was carried out for stomach diseases and hypertension continuously in Kamioka Town, Gifu Prefecture. The report for 1961 was published already as part 1.** This paper part 2 covers the years 1962 and 1963.

1. Stomach Diseases

The subjects of this examination over 40 years and their number was 1,024 in 1962 and 810 in 1963.

Among these subjects, 7 were found with suspected stomach cancer, 18 with stomach ulcer, 26 with duodenal ulcer, 83 with chronic gastritis, 103 with gastroptosis in 1962, and 11 with suspected stomach cancer, 1 with chronic gastritis, and 196 with gastroptosis in 1963.

The 18 patients suspected stomach cancer were diagnosed as chronic gastritis or stomach ulcer by operation or more exact examination. Thus there were no patients with stomach cancer, and only one case of esophageal cancer in these two years.

2. Hypertension

The number of subjects examined for hypertension was 1,266 in 1962, and 1,065 in 1963. All of them were over 30 years.

The percentage of those with systolic and diastolic hypertension increased with advance of age. When the subjects were classified according to Master's limits of blood pressure, the percentage of those with systolic hypertension began to increase at age 55 in the male and reached the maximum at 65. In the female it began to increase gradually at 40 and became a little larger than the male percentage at age 55 but smaller at 65. The percentage at 55 in the male. In the female it started to increase as early as at 40 and reached the maximum at 55.

The subjects with both hypertension and cardiac deformation revealed on the chest roentgenography began to increase at 66 in the male and at 55 years in the female.

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6. ACQUIRED IMMUNOLOGICAL TOLERANCE TO HUMAN SERUM

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One hundred and three rabbits were subjected to experiments to investigate the influence of neonatal injection of human serum (HS) on the production of precipitating antibody to HS. Except in Experiment 4, the HS used was from O-type blood.

Experiment 1. Four groups of rabbits — Group A (4 rabbits), Group B (5), Group C (7) and Group D (4) — were daily injected intraperitoneally with 0.2 ml of HS from birth for 21 days, 126 days, 112 days and 102 days, respectively. No animal of Group A showed antibody production when 131 days old, while all the rabbits of the other three groups were observed 3 days after the last injection to have developed a small quantity of precipitin. Seven rabbits — 2 of Group A, 2 of Group B, 1 of Group C and 2 of Group D — were challenged subcutaneously with 3 ml of HS twice at interval of 3 days from 187 to 237 days after birth. Three control rabbits, about 150 days old, receiving no pretreatment, were also challenged in the same way. The results showed that the rabbits treated neonatally with HS as stated above were partially tolerant to Hs though only to a slight extent.

Experiment 2. Twenty rabbits, intraperitoneally injected daily with 1 ml of HS from birth for 21 days, were divided into 3 groups; Group E (8 rabbits) received no additional injection before challenge, Group F (6 rabbits) were injected additionally by the subcutaneous route with 1 ml of HS twice from 28 to 47 days after birth, and Group G (6 rabbits) were injected additionally with 1 ml of HS in incomplete Freund's adjuvant by the intramuscular route only once from 28 to 33 days after birth. All the rabbits of the 3 groups were challenged intravenously with 1 ml of HS weekly for 3 weeks beginning 70 to 89 days after birth. Five control rabbits, about 90 days old, receiving no pretreatments, were challenged in the same way. The results of this experiment indicated that most of the rabbits treated neonatally were completely tolerant to HS.

Experiment 3. Five groups of rabbits received neonatal treatment with HS from birth as follows; Group H (6 rabbits), 8 ml in 8 days, Group I (3 rabbits), 32 ml in 16 days, Group J (2 rabbits), 27 ml in 30 days, and Group K (3 rabbits) and Group L (6 rabbits), 34 ml in 20 days. Groups H, I, J, K, L and 10 non-treated control rabbits were given the first challenge injection of 2 ml of HS weekly three times by the intravenous route 3 to 4 months after birth. It was observed that most of the rabbits of Group H were partially tolerant and all of Groups I, J and K were completely tolerant.

When 7 to 8 months old, the rabbits of Groups H, J and K received the second challenge injection in the same way. The data at this time represented that all the rabbits of Groups J and K were still tolerant but the tolerance retained by the animals of Group H varied all the way from nil to totality. Moreover, in Groups J and K, 4 of the 5 were still tolerant when 11 to 12 months old. The rabbits of Group L were first challenged 228 days after birth and most of them were found to be partially tolerant.

Experiment 4. In this part HS from O-type blood (HS_O) and HS from AB-type blood (HS_{AB}) were used as the antigen. Group M (3 rabbits) and Group N (2 rabbits) were daily injected intraperitoneally with 1 ml of HS_{AB} for 21 days from birth. Group M was challenged weekly with 2 ml of HS_{AB} three times beginning 98 days after birth and 4 rabbits of Groups E, F and G (cf. Experiment 2) were challenged in the same way beginning 163 to 177 days after birth, while Group N was challenged with HS_O beginning 91 days after birth. The rabbits treated neonatally with HS_O or HS_{AB} were observed to be tolerant to both HS_O and HS_{AB} when the challenge injection was given.

Experiment 5. Six adult rabbits, not treated neonatally, were taken. Three of them were injected daily with 20 ml of HS for 7 days. The other 3 rabbits were injected weekly with 2 ml of HS for 3 weeks. The antibody production in the former was a little higher in the latter.

Experiment 6. Four rabbits of Group E (cf. Experiment 2), treated neonatally and challenged later with HS, were challenged intramuscularly with 3 ml of a saline extract of stomach from a patient with gastric cancer (GCE) in incomplete Freund's adjuvant twice at intervals of 2 weeks from 163 to 191 days after birth. Three rabbits of Group I (cf. Experiment 3) receiving neonatal and later injections with HS were challenged with a saline extract of stomach from a patient with gastroptosis (NGE) in the same way. All of the 4 control rabbits, not treated neonatally and challenged later with NGE or GCE, produced anti-HS. But nine of the rabbits, receiving pretreatment with HS, were observed to produce anti-HS after the challenge injection with NGE or GCE.

From these experimental data it has been concluded :

- 1) Acquired immunological tolerance to HS is induced in rabbits receiving injections of 1 ml of HS daily for 21 days or of 2 ml of HS for 16 days from birth.
- 2) The tolerance induced by daily injection with 1 ml of HS for 8 days from birth was not complete but partial.
- 3) Tolerance to HS can be prolonged up to one year after birth by challenge injection given at certain intervals of time following the tolerance injection.
- 4) No difference of tolerance induced by neonatal injection of HS was observed whether it came from O-type blood or AB-type blood.
- 5) In adult rabbits not treated neonatally with HS the anti-HS production was not inhibited by daily injection of 20 ml of HS given for 7 days.
- 6) The production of anti-HS factor by injection of saline extracts of organs to adult rabbits can be inhibited by neonatal injection of HS.

7. DIFFERENCE BETWEEN SHEEP AND GOAT RED CELLS IN THE
AGGLUTINABILITY BY ANTI-FORSSMAN RABBIT SERUM

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Since Forssman (1911), it is a well known fact that although sheep red cells are highly susceptible to lysis in the presence of guinea-pig complement they have only low susceptibility to agglutination by the Forssman rabbit serum (anti-F). In his serological study, the author observed that goat red cells are completely resistant to agglutination although highly sensitive to lysis by the serum, and therefore carried out a series of experiments in order to clarify the reason of the difference in agglutinability between sheep and goat red cells.

The results showed that the difference of agglutinability between sheep and goat red cells apparently depended upon the difference of the antigen situation, that is, the Forssman antigens of goat red cell are situated at such a depth that even a bivalent anti-F, after combining with an F antigen in one cell, is of insufficient length to link across to the F antigen in a second cell. On the other hand, although the majority of the F antigens on sheep red cells lie at such a depth as those observed in goat red cells, some of them lie more superficially, and as a consequence agglutination can be caused by the serum though only to a small extent. Moreover, this antigen situation in sheep and goat red cells may be one of the reasons that make them highly susceptible to lysis and relatively resistant to agglutination by the serum.

IN
8. POSSIBLE PRESENCE OF RNA PEPTONE PREPARATIONS AS
DEMONSTRATED BY STREPTOLYSIN-S FORMATION INDUCING EXPERIMENT

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In the course of the study on the RNA-effect in inducing streptolysin-S formation, an observation was made that when washed hemolytic streptococci were incubated in phosphate-buffered Ringer's solution containing 3% commercial peptone, the medium acquired high hemolytic activity within 2 hours.

On the basis of this finding, a series of experiments concerning the influence of peptone on hemolysin formation by resting cells of hemolytic streptococci and the properties of the hemolysin were performed.

The principal results may be summarized as follows :

1) The amount of hemolysin formed is a function of the quantity of peptone added to the resting cell system medium, i.e., phosphate-buffered Ringer's solution; although the effect of peptone varied with different preparations of commercial peptone, with 5% concentration of a peptone preparation, the supernatant, peptone-induced hemolysin, exhibited hemolytic activity up to a dilution of 1:1,000.

2) The effect of peptone in causing enhanced hemolysin formation appears to be specific for β -hemolytic streptococci, since comparable phenomenon was not observed in experiments carried out with other species of bacteria, such as *Str. viridans*, *Staphylococcus*, *Pneumococcus*, *Corynebacterium diphtheriae*, *Cl. Welchii* and *E. coli*.

3) The hemolysin formed in the medium has following additional properties :

- a) Its hemolytic activity is completely antagonized by trypan blue.
- b) It is not activated by cysteine.
- c) It is thermo-labile, but oxygen-stable.
- d) It is non-dialysable.

4) There was, moreover, a parallelism between the results obtained in experiments with peptone and those obtained in experiments with yeast ribonucleic acid.

5) Thus, it was thought that these results constitute strong evidence for the identity of the peptone-induced hemolysin with the RNA-induced streptolysin-S and for the existence of a minute amount of RNA having potent streptolysin-S inducing activity in commercial peptones.

Further, it is to be noted here that Y. Taketo of our laboratory has recently succeeded in isolation of ribonucleotide fraction having very potent streptolysin-S inducing activity from non-dialysable part of commercial peptone (*Japan. J. Exp. Med.*, **33**, 33, 1963).

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

- 1) Okamoto, H., Fujimura, A., Hayashi, T., Nishida, N., Shimizu, R. and Koshimura, S.: Experimental Anticancer Studies. XX. Effect of Live Hemolytic Streptococci, Grown in Ribonucleic Acid-containing Broth, on Tumor Cells. Gann, **55**, 225-232, 1946.

The tumor cell-damaging activity of live hemolytic streptococci grown in the following five culture media was investigated: (1) 0.8% RNA broth, (2) 0.8% RNase-core broth, (3) plain broth, (4) 0.8% glucose broth, and (5) 0.8% RNA-0.8% glucose broth.

In parallel with these anticancer experiments, comparative experiments were also made on the streptolysin-S producing ability of the cocci grown in these different media.

1) Indications were that hemolytic streptococci grown in 0.8% RNA (or RNase-core) broth are very effective (more so than those grown in plain broth) in causing loss of the invasion power of Ehrlich ascites carcinoma cells in mice.

2) Additional experiments have shown that hemolytic streptococci grown in 0.8% glucose broth and in 0.8% RNA-0.8% glucose broth were entirely ineffective.

3) Data suggesting that there is a close relationship between the streptolysin-S producing ability and tumor cell-damaging capacity of hemolytic streptococci were also presented.

- 2) Koshimura, S., Shimizu, R., Fujimura, A. and Okamoto, H.: Experimental Anticancer Studies. XXI. Effect of Penicillin Treatment of Hemolytic Streptococcus on Its Anticancer Activity. Gann, **55**, 233-236, 1964.

A series of diluted streptococcal suspensions were prepared and incubated at 37°C for 20 minutes after addition of penicillin solution. Anticancer activity was compared in the penicillin-pretreated and non-treated cocci suspensions. It was found that *in vitro* pretreatment of hemolytic streptococci with penicillin caused a marked increase in the ability to deprive Ehrlich ascites tumor cells of their invading power in mice.

The penicillin-pretreated cocci were found to be as effective as the original cocci in streptolysin-S formation, tested in the resting cell system.

- 3) Okamoto, H., Fujimura, A., Shoin, S., Koshimura, S., Bando, I. and Ujiie, T.: Studies on the Phenomenon of High Promotion by Nucleic Acid of the Production of Streptolysin-S of Hemolytic Streptococcus. Part 22. On the Inhibition by Glucose of Streptolysin-S Production in Ribonucleic Acid Broth Culture. Japan. Exp. Med., **34**, 109-118, 1964.

The antagonistic phenomenon between glucose and ribonucleic acid in the streptolysin-S formation by hemolytic streptococci was further studied.

The experimental data are presented to show that streptolysin-S production was definitely inhibited by the addition of glucose in concentrations of 0.5-1.0% to the culture media containing 0.8% RNA (or RNase-core), and that the cocci, which had grown in the culture media containing 0.8% glucose, were tested, in the resting cell system, to have almost lost their ability to produce streptolysin-S.

- 4) **Koshiura, R., Koshimura, S. and Bando, Y.** : Experimental Anticancer Studies. XXIII. Further Experiments on the Antitumor Activity of 4-Amino-6-octylresorcinol Hydrochloride. Gann, **55**, 433, 1964.

4-Amino-6-octylresorcinol hydrochloride (AOR) was further investigated for its antitumor activity on some transplantable mouse and rat tumors : Ehrlich ascites carcinoma, Sarcoma 180, Leukemia SN 36, Yoshida sarcoma and Ascites hepatoma AH 13.

AOR was effective not only in inhibiting the growth of both the ascites and solid forms of Ehrlich ascites carcinoma 180, but also in inhibiting the growth of Ascites hepatoma AH 13. However, this compound did not show any antitumor activity on Lymphatic leukemia SN 36 and Yoshida sarcoma.

- 5) **Tada, S.** : Hemophilic Arthropathy. Rinsho-hoshasen, **2**, 242, 1964.

Two cases of osseous changes in siblings with hemophilia A are presented, with a special reference to the X-ray picture.

- 6) **Tada, S., Kido, T. and Takata, H.** : Endogenous Lipid Pneumonia. Rinsho-hoshasen, **9**, 975, 1964.

Two cases of endogenous lipid pneumonia are reported; one is considered to be essential and the other secondary to bronchiectasis according to Inada's classification.