STUDIES ON AN ONCOSTATIC AGENT "K. C. G."

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The oncostatic agent "K. C. G." is a metabolic product of *Serratia marcescens*, containing bacterial polysaccharide.

Coley, W. B. (1894) employed crude filtrates from two species of bacteria in the treatment of cancer patients. *Serratia marcescens* was one of them. This subject was reviewed by Shear, M. J. (1936). Shear and his colaborators began to purify the factor in the bacterial filtrates and developed a satisfactory bioassay method. They isolated a highly active tumor-necrotizing material from filtrates of culture of *Serratia marcescens*. Paul Rathgeb, & Bengt Sylven (1954) reported experimental results on the tumor-necrotizing agent from *Serratia marcescens*. This agent is called Shear's Polysaccharide, which produces hemorrhagic necrosis in tumors.

Recently, the author has studied on adaptation of *Serratia marcescens* under Prof. Ashida, J. of Kyoto University, and succeeded to induce the polysaccharide-rich mutant strain by cadmium treatment in 1958. The metabolic product of this mutant strain of *Serratia marcescens*, the oncostatic agent "K. C. G.", inhibits tumor-cell division and necrotizes tumor-tissue, and moreover, largely unaffects on normal tissue. It is interesting problem that "K. C. G." has strong effect on tumor and little effect on normal tissue. These experimental results were observed in clinical field as well as animal research.

MATERIALS AND METHODS

The original strain of *Serratia marcescens* was derived from the pus of a surgical patient at Bacteriological Laboratory of Osaka Medical College in 1952, and has been cultured in Kayama Laboratory of Biological Institute, Wakayama University. In 1958, the mutant strain of *Serratia marcescens* which could richly produce polysaccharide was induced from the original strain by cadmium treatment as mentioned above. Bacteria of this mutant strain were inoculated in the synthetic liquid medium, and after being cultured for 24 hours at 30°C, bacteria were removed by filter tube. Next, the bacterial filtrates were dialyed with cellophane, and so all salts that had till then remained in the filtrate were completely removed, and bacterial products containing polysaccharide were remained in the filtrate. After dialysis for 36 hours, the filtrate was dried up by freezing to obtain the white powderlike oncostatic agent K. C. G.". This agent was dissolved in distilled water or "Moriamin S" for injec-

tion as animal and clinical researches.

Mice of DD-strain and Ehrlich ascites tumor were employed in these experimental researches and rabbits were also used in histological and hematological researches.

RESULTS

(1) Chemical Properties

The chemical properties of "K. C. G." are as follows. It is white powder and is decomposed at about 152°C with a tendency of becoming brown-colored. It does not have the property of sublimation. It contains carbon, hydrogen, oxygen, nitrogen, and phosphor as its constituent elements; it is insoluble in hexan, benzene, ether, methanol, ethanol, butanol, ethyl acetate, carbon tetrachloride, etc., and is hardly soluble in acetone, dioxan, and acetic acid, but is soluble in water, and completely soluble in "Moriamin S".

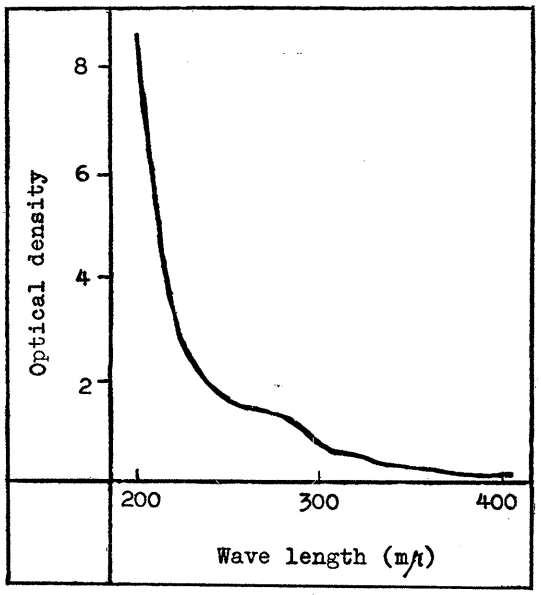


Fig. 1 Spectral absorption of K. C. G.

In the qualitative reaction, it is negative against the Fehling reaction silver mirror reaction, burrette reaction, resorcinol reaction and ferric chloride reaction, but it is positive against the ninhydrin reaction and assumes a purplish red color. Those hydrolyzed with 6-normal hydrochloric acid are positive against both the Fehling reaction and the silver mirror reaction.

When measured with a Beckmann's Quarz spectrophotometer, it shows a strong absorption zone at the vicinity of 200 mμ and a weak absorption zone at the vicinity of 265 m.

(II) Toxicity

1) L. D. 50

In the case of normal mice, L. D. 50 was 4900 mg/kg of body weight as subcutaneous injection and 1175 mg/kg as intraperitoneal one. It was proved that toxicity of "K. C. G." was extremely weak.

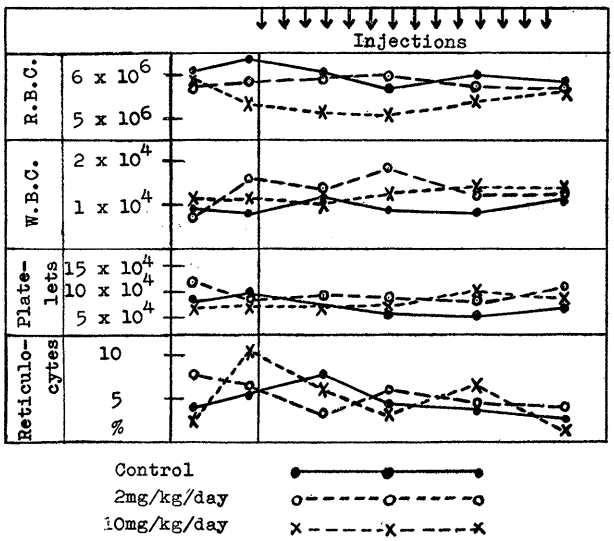


Fig. 2 Hematological effects of K. C. G., in the case of rabbit (1)

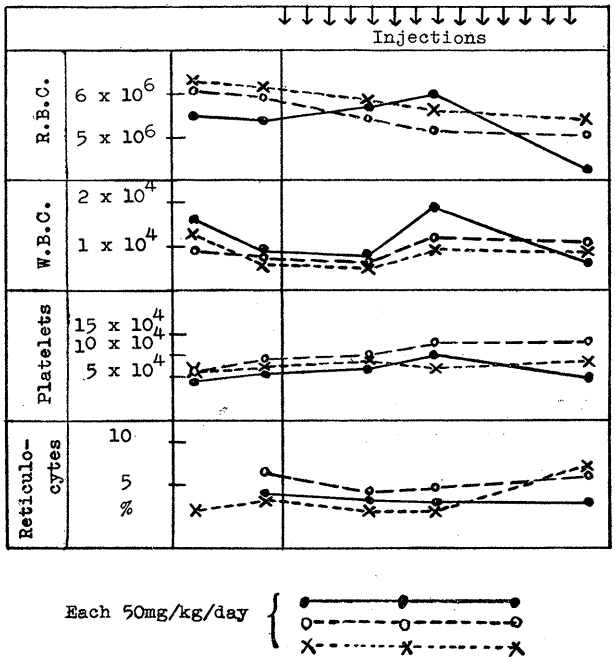


Fig. 3 Hematological effects of K. C. G., in the case of rabbit (II)

2) Hematological Effect

Various dosages of "K. C. G." were subcutaneously injected every day for 2 weeks in normal rabbits. These results are shown in Fig. 2, & 3.

From these experimental results, it was proved that "K. C. G." has little hematological effect in the case of rabbit.

Especially, it was noteworthy results that leukopenia was not caused by "K. C. G." even in the case of repeated injections of great amount of dosage as 50 mg/kg.

3) Pyrogenic Reaction

Various dosages of "K. C. G." were subcutaneously or intraveneously injected in normal rabbits. Body temperature began to rise up usually from 1 or 2 hours after injection of "K. C. G." and fall down within 5 or 6 hours. (Fig. 4)

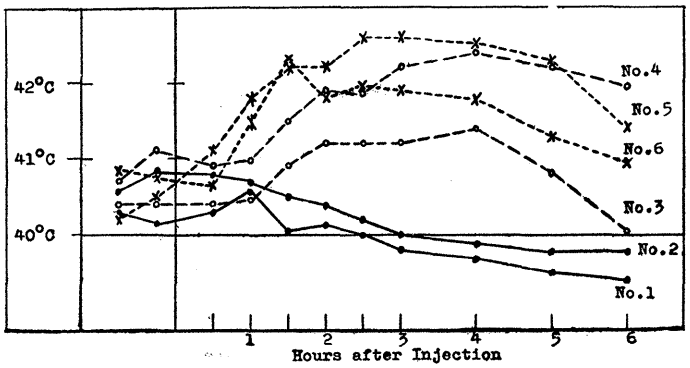


Fig. 4 Pyrogenic reactions of K. C. G., in the case of rabbit

No. 1 •—•—• Control (intravenous injection of distilled water)
No. 2 •—•—• Control (subcutaneous injection of distilled water)
No. 3 ∘– –∘– –∘ } Subcutaneous Injection (K. C. G. 50 mg/kg/day)
No. 4 ∘– –∘– –∘ }
No. 5 ×···×···× } Intravenous Injection (K. C. G. 50 mg/kg/day)
No. 6 ×···×···× }

It was proved that "K. C. G." was also pyrogenic substance as other bacterial polysaccharide.

4) Hstological Effect on Rabbits

Various dosages of "K. C. G." were subcutaneously injected every day for 2 weeks in normal rabbits. These histological effects are shown in Table 1.

5) Histological Effects on Mice

Various dosages of "K. C. G." were subcutaneously injected every day for a week in normal mice.

These histological effects are shown in Table 2.

From these experimental results, it may be said that the lung and the genital gland were effected remarkably by "K. C. G." in mice as well as rabbits. (Fig. 5)

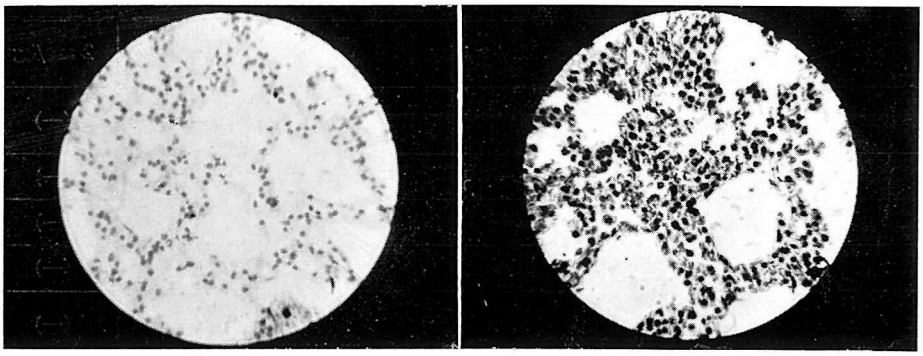
Table 1. Historical effects of K. C. G. in rabbit.

Tissue \ Dose	50 mg/kg/day	10 mg/kg	2 mg/kg
Liver	Slight degeneration of cytoplasm, unaffected on nuclers	(—)	(—)
Kidney	(—)	(—)	(—)
Heart	(—)	(—)	(—)
Lung	Hyperemia & edema of alveolar walls	(—)	(—)
Spleen	Slight herorrhage & hyperemia of red purps with deposit of hemosiderins	(—)	(—)
Pancreas	(—)	(—)	(—)
Testis	Atrophy of spermatic duct especially germ cells, unaffected on Sertoli's cells & Leydig's cells	Same as 50 mg/kg	Same as 50 mg/kg
Thyroid gland	Hypofunction such as diminished follicles & scanty colloid storage, in some cases pancreaslike glandular structure with hydropic degeneration of epitheliums	Same as 50 mg/kg	Same as 50 mg/kg
Adrenal gland	(—)	(—)	(—)
Digestive duct	(—)	(—)	(—)
Cerebrum	(—)	(—)	(—)

Table 2. Histological effects of K. C. G. in normal mice

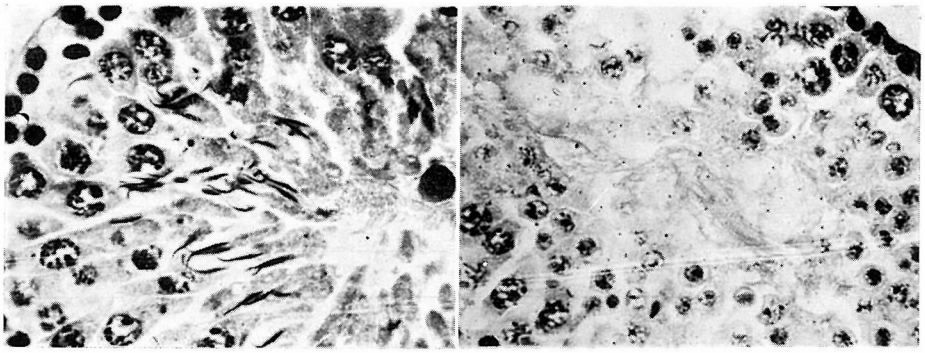
Tissue \ Dose	200 mg/kg/day	20 mg/kg/day
Liver	Remarkable vacuolation	Slight vacuolation
Heart	(—)	(—)
Kidney	(—)	(—)
Lung	Remarkable edema & hyperemia in alveolar wall	(Same)
Genital gland	Remarkable atrophy no sperm contraction of ovarium	Sporadic atrophy
Suprarenal	Hyperemia	(—)
Cerebrum	(—)	(—)

Alveolar walls of lung



Control K. C. G. -Treatment

Genital gland (♂)



Control K. C. G. -Treatment

Fig. 5 Histological effect of K. C. G. in mice K. C. G.-Treatment: (200 mg/kg/day, for a week, subcutaneous injection)

(III) Oncostatic Activity for Ehrlich Ascites Tumor

1) Effect on Ehrlich Ascites Tumor in Mice by Intraperitoneal Injection

Ehrlich ascites tumor cells (about 8,000,000 cells) were transplanted in abdominal cavity of male mice (NA_2-Strain). These mice were devided into three groups. The first group was control. The second group was treated by "K. C. G." (0.05 mg/1 mouse/day). The third group was treated by "K. C. G." (0.1 mg/1 mouse/day). Each group consisted of 3 mice, and average values among 3 mice were shown in Fig. 6. (1n every cases, "K. C. G." treatments were tried by intraperitoneal injections repeatedly for a week.)

From these results, it was confirmed that body weights of lst group-mice were increased about 23.1 % compared with their initial weight, but on the contrary, body weight of 2nd and 3rd group-mice were not increased. It is considered that the increase of body weight results from the stagnation of ascites, and so it is clear that "K. C. G." inhibit the stagnation of ascites.

2) Effect on Ehrlich Solid Tumor in Mice by Intraperitoneal Injection

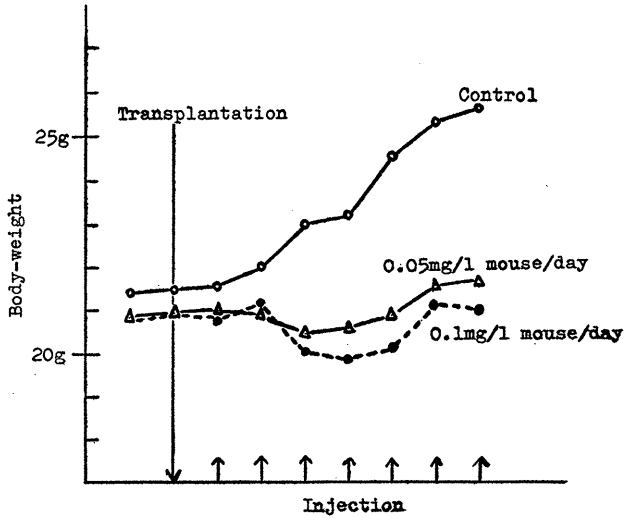


Fig. 6 Body weights of tumorbearing mice

○—•—• Control
△—△—△ 0.05 mg
•···•···• 0.1 mg } K. C. G. Treatment (/1 mouse,/day)

Ehrlich ascites tumor cells (about 16,000,000 cells) were transplanted subcutaneously on dorsal side of male mice (DD-Strain). These mice were devided into two groups. One of them was control, and the other was treated by "K. C. G." (5mg/1 mouse, every day, for a week).

At 8 days after transplantation, the solid tumors were taken up from their body, and their fresh weight were weighed.

These results are shown in Table 3 and Fig. 7.

From the experimental results, mentioned above, it was proved that "K. C. G." had remarkable oncostatic activity for Ehrlich solid tumor.

(IV) Degenerative Activity for Ehrlich Ascites Tumor Cell

The mice (NA_2-Strain) were injected "K. C. G." (dosage 1 mg/1 mouse) at 72 hrs after transplantation of tumor cell.

Table 3 Effect of K. C. G. on Tumor-Weight

	Average %	Tumor-Weight (mg)								
Control	100	2670	1620	1290	1260	1190	1100	960	705	695
Treatment of "K. C. G."	40•4	900	720	610	510	510	420	405	320	250

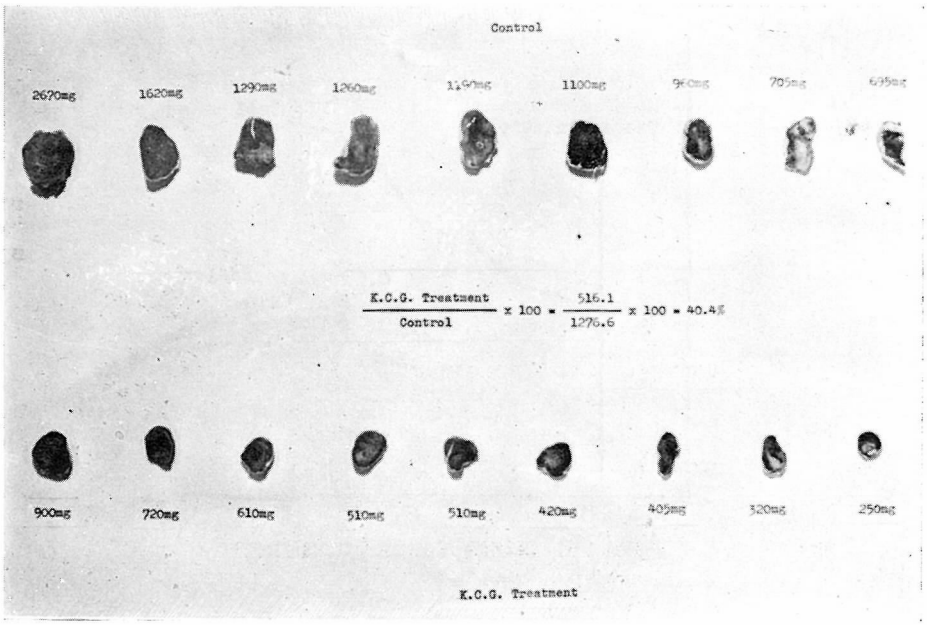


Fig. 7 Oncostatic effects of K. C. G.

Table 4 Degenerations in Ehrlich ascites tumor cells by single intraperitoneal injection of K. C. G.

Hours after injec. K. C. G.	0	1	3	6	9	12	24
Total Cells	953	871	557	709	731	773	795
Division Cells	55	34	29	8	20	18	18
Prophase	12	9	9	2	4	5	9
Metaphase	22	6	9	2	10	9	6
Anaphase	12	12	5	4	3	2	0
Telophase	9	7	6	0	3	2	3
Rate of Division Cell	5%8	3.9	5.2	1.1	2.7	2.3	2.3
Abnormal Cells	5	35	118	195	191	213	393
Rate of Abnormal Cell	0%5	4.0	21.7	27.5	26.1	27.6	49.4

It was proved in these results that "K. C. G." had degenerative effects on Ehrlich tumor cells. It was interesting problem that mitosis of tumor cell was inhibited by "K. C. G." and abnormal cells were increased with the lapse of time.

(V) Life-Prolongation of Tumor-bearing Mice by "K. C. G." Treatment

1) Intraperitoneally Injection

Ehrlich ascites tumor cells (about 8,000,000 cells) were transplanted in abdominal cavity of male mice (NA_2-Strain). These mice were devided into three groups. The first group was control. The second group was treated by "K. C. G." (dosage,

0.05 mg/1 mouse). The third groud was treated by "K. C. G." (dosage, 0.1 mg/1 mouse). In every cases, "K. C. G." treatments were tried by intraperitoneal injections repeatedly every day for a week from 24 hrs after transplantation.

In the cases of the first group (control) and the second group, all mice died at 12 days—14 days after transplantation. On the contrary, in the case of 3rd group, 50% of treated mice survived over 3 weeks. And some of them remained health and free from recurrence.

2) Subcutaneous Injection

Ehrlich ascites tumor cells (about 8,000,000 cells) were transplanted in abdominal cavity of male mice (NA_2-Strain). These mice were devided into three groups. The first group was control. The second group was treated by "K. C. G." (dosage, 0.1 mg/1 mouse). The third group was treated by "K. C. G." (dosage, 1 mg/1 mouse). In every cases, "K. C. G." treatments were tried by subcutaneous injections repeatedly every day for a week from 24 hrs after transplantation.

In these experiment survival ratio of the 3rd group's mice amounted to 50%, and in the case of 2nd group, survival ratio amounted to 25%.

DISCUSSION

From various experimental results mentioned above, it proved that "K. C. G." has remarkable oncostatic activities, and has little toxicities.

"K. C. G." is a metabolic product of *Serratia marcescens*, containing bacterial polysaccharide. Shear, M. J. employed also bacterial polysaccharide extracted from the filtrates of *Serratia marcescens* as an oncostatic agent. There were great differences between "K. C. G." and Shear's Polysaccharide, in regard to toxicity. Toxicity of "K. C. G." was extremely weak. This fact was one of the favorable characters of "K. C. G.". It may be considered that differences of toxicity between "K. C. G." and Shear's Polysaccharide were caused by Mg-effects. Shear had reported that degenerative activities of bacterial polysaccharide for tumor cells were weakened by Mg in culture medium. The author considered that Mg which weakened degenerative activity for tumor cells could also weaken degenerative activity for normal cells. Diminution of degenerative activity for normal cells means reduction of toxicity. From the reason mentioned above, toxicity of "K. C. G." was extremely weak, and it may be said that the mutant strain of *Serratia marcescens* which has little toxicity was induced by Mg.

The most valuable character of "K. C. G." as anti-cancer drug was effectless for hematogenic function. Especially leukopenia was never caused by "K. C. G.". This fact was favorable to use "K. C. G." clinically for a long term.

"K. C. G." was pyrogenic substance, and so pyrogenic reactions were caused in every case of clinical experiment as well as animal experiment. It was a well-known fact that bacterial polysaccharide had pyrogenic substance, for instance T. T. G. which was Pseudomonas Polysaccharide had also Pyrogenic substance. Coley, W.

B. and Shear, M. J. indicated that metabolic product of *Serratia marcescens* had pyrogenic substance. It may be unfavorable factor that "K. C. G." has pyrogenic substance, but usually these pyrogenic reactions disappeared within several hours. Thus, these pyrogenic reactions should be considered as physiological phenomena, and should be taken no notice in the clinical field.

From histological experiments of these research, it was proved that "K. C. G." effected remarkably on genital glands (testis or ovarium) and also on alveolar walls of lungs, in mice as well as rabbits. But their effects in histological field were only caused when "K. C. G." were used in extreme large dosages. Thus, there was nothing to fear in clinical experiment about these histological effects of "K. C. G.".

It was proved that "K. C. G." has degenerative effects for Ehrlich ascites tumor cells. These degenerative effects of "K. C. G." for tumor cell were also proved by Dr. Shiomi. F. and others in the case of Yoshida sarcoma in rat, and also proved by Dr. Kimura, T. in the case of *Peritonitis carcinomatosa* in man.

From the various experimental results mentioned above, it was confirmed that "K. C. G." was very valuable anticancer drug, because it has strong oncostatic activities and has little toxicity. In July 1960, the first clinical study was made by Dr. Hishikawa, Wakayama City. He tried this agent to a severe case of metastatic bone cancer originated from breast, in which a surprising effect was observed both clinically and roentgenographically. (Fig. 8) (43 age ♀)

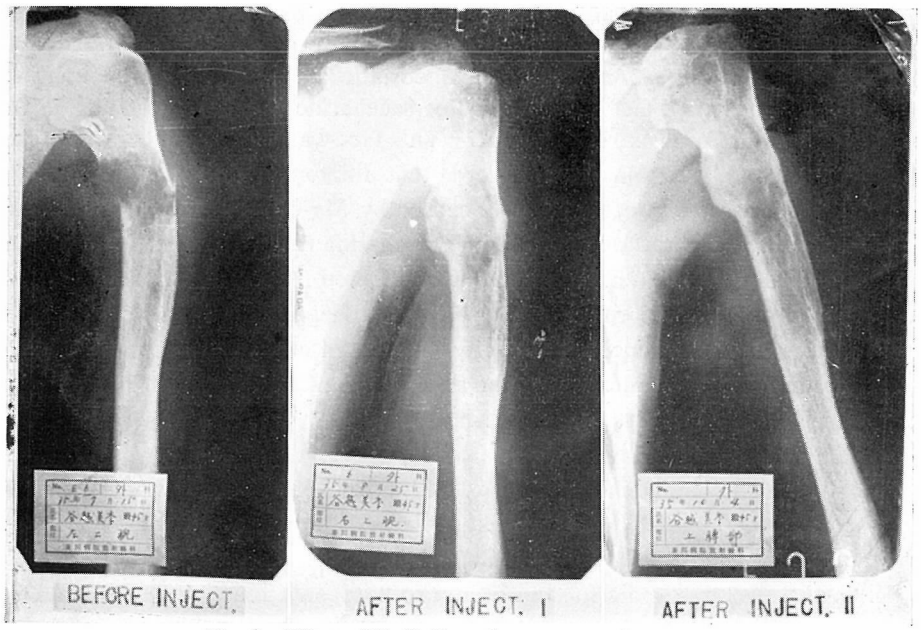


Fig. 8 Effect of K. C. G. on bone metastatic cancer

Since, this first clinical experiment, "K. C. G." has been employed at various

hospital in Japan. These clinical results will be reported by many researchers in future.

SUMMARY

1. An oncostaaic agent "K. C. G." was a metabolic product of *Seratia marcescens*, containing bacterial polysaccharide.

2. The polysaccharide rich mutant strain of *Serratia marcescens* induced by Cd-treatment.

3. Toxity of "K. C. G." was extremely weak, especially in the hematological field. Leukopenia was never caused by "K. C. G."

4. Pyrogenic reactions were observed in clinical experiments as well as animal experiments.

5. In the histological researches, it was proved that "K. C. G." did effect on alveolar wall of lung and genital gland.

6. Oncostatic activities of "K. C. G." were confirmed on the Ehrlich ascites tumor (ascitic & solid) in mice.

7. It was proved that "K. C. G." had degenerative activity for Ehrlich tumor cell remarkably.

8. Life-prolongation of tumor-bearing mouse by K. C. G.-treatment was observed.

9. In July, 1960, the first clinical experiment was made by Dr. Hishikawa, Wakayama City. He tried this agent to a severe case of metastatic born cancer originated from breast, in which a surprising effect was observed both clinically and roentgenographically.

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