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Studies on the Carcinogenic Effect of Actinomycin*

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SUMMARY

Subcutaneous administration of actinomycin S and L to various stocks and strains of mice produced sarcomas. These sarcomas were transplantable for many generations. Although carcinostatic activity of actinomycin L is about one third of that of actinomycin S, the carcinogenic activity of actinomycin L is about one tenth of that of actinomycin S. The possible mode of action of actinomycin causing carcinogenesis in mice is discussed.

INTRODUCTION

Actinomycin is an antibiotic which was first reported by Waksman and Woodruff (1940). It was soon discovered that there are several types of actinomycin. Most of them are mixtures of different types as shown by paper chromatography and counter-current distribution techniques. Brockmann and Grubhofer (1949) showed that actinomycin is a peptide attached to phenoxazone moiety and this phenoxazone was suggested to be common to all of actinomycin examined (Brockmann and Vohwinkel, 1956). However the nature, number and sequence of amino acids of different actinomycins vary. The chemical structure of actinomycin C was determined by Brockmann and his co-workers (1956). Bullock and Johnson (1957) determined that of actinomycin D.

Though actinomycin is a powerful antibiotic, it is also highly toxic to animals. Waksman *et al.* (1941) and Robinson and Waksman (1942) studied the effect of actinomycin A on the spleen and lymph nodules of experimental animals. Since the discovery of the cytostatic activity of actinomycin C (Hackmann, 1952), several investigations have been made on its antitumor effect (Hackmann, 1952, 1954; Schulte, 1952; Schulte and Lings, 1953; Shiba, 1956; Reily *et al.*, 1953; Umezawa, 1955; Hackmann and Schmidt-Kastner, 1957). If actinomycin has true antitumor activity, it might produce tumors in experimental animals after long term administration. In the previous paper, Kawamata *et al.* (1958) briefly reported on the carcinogenic effect of actinomycin. In this paper results are given on the carcinogenic effect of actinomycin S and L in various stocks and strains of mice.

* An outline of this work was reported at the 17th General Meeting of the Japanese Cancer Association in Chiba City in November, 1958.

MATERIALS AND METHODS

Actinomycin:* In all experiments the same batches of actinomycin S and L were used. All these drugs were prepared from the mycellium of streptomyces. These actinomycins both contained four components two of which were major components. Some chemical and biological characteristics of these actinomycins have been reported by Kawamata and Fujita (1958 a, b) and by Fujita (1959).

Experimental animals: Mice of the following stocks and strains were used: btk, ctk, C3H/MsMa, C57BL/De, Swiss albino/Ma and ddO. The origins and histories of btk and ctk stocks are given in the previous paper (Kawamata *et al.*, 1958). All animals except stock ddO were bred in our own laboratory. Stock ddO was supplied from the Central Breeding Station of Experimental Animals, Osaka University.

Ten animals were used in each group of experiments. Mice, in groups of five to ten, were housed in aluminum cages. Shavings, sterilized at 120°C. for 20 minutes, were used for bedding. The room temperature was maintained at 22–25°C. The mice were fed with mouse pellets "MF" made by Oriental Yeast Co. Ltd. and water *ad libitum*. Toward the end of the experiment, animals developing lesions were placed in individual cages.

Preparation of solution of actinomycin for injection: To prepare the solution of actinomycin, crystals of actinomycin were dissolved in a minute amount of ethanol (99%). This was then diluted with saline and sterilized with a Seitz filter. The filtrate was sealed aseptically in glass ampules each containing 50 μg. in 2 ml. aliquots. The ampules were kept in a refrigerator at 5°C.

Injection of the drug: 0.1–0.4 ml. of the solution from the ampule diluted with sterile saline solution to an appropriate concentration, depending on the amount of the drug used for injection, was administered subcutaneously into the right interscapular region of the mice, using a 1/4 gauge needle and syringe. Administrations were repeated twice a week until signs of tumor production developed. Two control groups were also prepared. The one group receiving no treatment and the other was injected with saline solution containing the same amount of ethanol as the actinomycin solution. The control animals was examined after 40 weeks. If no sign of tumor production was observed in the experimental group, injections of the drug were continued for one year.

Histological methods: For routine purposes, tissues were fixed in formol and paraffine sections were stained with hematoxylin and eosine.

Methods of transplantation of tumors: Small pieces of tumor, excised aseptically, were transplanted into the subcutaneous tissue or intraperitoneal cavity without the application of any antibiotics.

RESULTS

Induction of tumors in various stocks and strains of mice:

As shown in Table 1, after injection with 7.5 μg./kg. actinomycin S all nine or nine of ten btk mice developed histologically proved sarcomas. Control mice showed no sign of tumor production. Administration of actinomycin L proved less effective than actinomycin S in producing tumors in mice. Injection of as much as 67.5 μg./kg. of actinomycin L produced sarcoma in five of eight mice. Injection of 7.5 μg./kg. actinomycin S produced sarcomas in all nine male and seven of eight female ctk mice. Only one of ten male and one of eight female Swiss albino mice developed after the administration of the same doses of actinomycin S. In ddO mice sarcoma was induced in four of eight male and four of ten female animals. There was no sign of sarcoma production in C3H/MsMa mice. Injec-

* Actinomycin S was reported as actinomycin 1048A and actinomycin L as actinomycin 2104L respectively in the previous papers.

Table 1. Tumor production by actinomycins in various stocks and strains of mice.

Stock or Strain	Sex	Injection	Dose (μg./kg. or ml.)	Appearance (Weeks)	Incidence ratio
btk	♀♂	Actinomycin S	0.94	39	2/8
〃	〃	〃	〃		0/7
〃	〃	〃	7.5	21	9/10
〃	〃	〃	〃	25	9/10
〃	〃	〃	〃	19	9/9
〃	〃	〃	〃	20	7/10
〃	♀	〃	15.0	20	7/8
〃	〃	〃	30.0	16	10/10
〃	〃	〃	125.0	19	2/6**
〃	〃	Actinomycin L	9.0		0/8
〃	〃	〃	22.5		0/5
〃	〃	〃	67.5	35	5/8
〃	〃	〃	250.0		0/5***
〃	〃	Solvent	0.1 ml.		0/7
〃	〃	〃	0.4 ml.		0/9
〃	♂	〃	0.1 ml.		0/9
〃	♀♂	None			0/7
〃	〃	〃			0/9
〃*	♀	Actinomycin S	7.5	19	7/10
〃	〃	〃	〃	16	8/9
〃	♀♂	Solvent	0.1 ml.		0/10
〃	〃	〃	〃		0/6
〃	♀♂	None			0/9
〃	〃	〃			0/10
ctk	♀	Actinomycin S	7.5	25	9/9
〃	〃	〃	〃	20	7/8
〃	〃	Solvent	0.1 ml.		0/7
〃	♀♂	None			0/9
Swiss albino	〃	Actinomycin S	7.5	26	1/10
〃	♂	〃	〃	40	1/8
〃	〃	Solvent	0.1 ml.		0/6
〃	♀♂	None			0/6
ddO	♀♂	Actinomycin S	7.5	27	4/8
〃	〃	〃	〃	18	4/10
〃	〃	Solvent	0.1 ml.		0/9
〃	♀♂	None			0/10
C3H	♀♂	Actinomycin S	7.5		—+
〃	〃	〃	〃		0/4
〃	〃	Solvent	0.1 ml.		0/2
〃	♀♂	None			—+
C57BL	〃	Actinomycin S	7.5	37	3/9
〃	♂	〃	〃	49	2/9
〃	〃	Solvent	0.1 ml.		0/3
〃	♀♂	None			0/11++

5 weeks old btk mice were used. Other stocks and strains were 10 weeks old. The "incidence ratio" shows the number of mice in which tumors were produced/number of mice still alive when first tumor was observed.

The ratios of controls are the result after 40 weeks.

* 5 weeks old mice.

** Because of the toxicity, mice received only nineteen injections.

*** Because of the toxicity, mice received only eighteen injections.

\+ C3H mice died during the course of experiment.

++ In this group five mice were used.

tion of 7.5 μg./kg. of actinomycin S produced sarcoma in three of nine male and two of nine female C57BL/De animals.

The latent period of tumor production:

When 10 week old btk mice received 7.5 μg./kg. actinomycin S, the earliest appearance of tumor was observed after 19 weeks. When 30 μg./kg. of antibiotic was adminstered, tumors developed after 16 weeks. When 5 week old btk mice received 7.5 μg./kg. actinomycin S, females produced tumors in 16 and male in 19 weeks respectively. 10 week old btk mice injected with 67.5 μg./kg. actinomycin L produced sarcamas only after 39 weeks. The latent periods in 10 week old ctk female and male mice were 20 and 25 weeks respectively, when 7.5 μg./kg. actinomycin S was given. Production of tumors in 10 week old Swiss albino female and male mice were observed after 40 and 25 weeks respectively. In female ddO mice tumors were produced after 18 weeks and 27 weeks in male animals. In C57BL/De mice, production of tumors were observed in females after 49 weeks and in males after 37 weeks.

Description of tumors:

In all animals the lesions were restricted to the subcutaneous tissue at the site of injection. Prior to the development of a typical sarcoma, localized thickening and rigidity was observed around the site of injection. At an advanced stage, the sarcoma appeared as a massive tumor with a definite rim ranging in size from 0.8 to 3.0 cm. in diameter. Thinning and graying of hair was often observed at the site of the lesion. (Fig. 1, Fig. 2, Fig. 3)

Histologically these tumors showed the characteristics of sarcomas with many typical mitotic figures and giant cell formation. (Fig. 4, Fig. 5, Fig. 6, Fig. 7)

No differences in the histological figures of tumors of different stocks and strains of mice could be found. (Fig. 8, Fig. 9)

Transplantation of tumors and metastasis:

As shown in Table 2, the transplantation of the tumors from btk to btk or

Table 2. Serial subcutaneous transplantotions of the tumor.

Strain * of tumor	Strain of mouse		Generation of transplantation	Number of mice receiving transplants				
	Tumor produced	Tumor transplanted		Total	Valid	+	−	Rate (%)
T 1	btk	ctk	1 — 21	93	91	88	3	96.7
T 3	〃	btk	1 — 18	109	108	100	8	92.6
T 7	〃	〃	1 — 18	87	77	65	12	84.4
T'1	ctk	ctk	1 — 31	138	133	130	3	97.7
TA	btk	btk	1 — 17	80	80	78	2	97.5
TG	ctk	ctk	1 — 2	9	8	6	2	75.0
TK	ddO	ddO	1 — 13	65	65	60	5	92.3
TN	Swiss albino	Swiss albino	1	2	2	2	0	100.0
TM**	btk	btk	1 — 13	45	44	43	1	97.7
TO	C57BL	C57BL	1 — 7	14	14	14	0	100.0

* Tumors from strain T 1 to T'1 were those produced in mice during the course of experiments reported in the previous paper (Kawamata *et al.*, 1958). Those from strain TA to TO were obtained in experiments reported here.

** This tumor was produced after treatment with a single dose of 125 μg./kg. of actinomycin S.

from ctk to ctk, that is in the same stock mice, was successful. The same specificity was observed among other stocks and strains. T'6 tumor, which is produced in ctk females could not be transplanted to ddO, C57BL/De and C3H/MsMa mice but was viable in btk and Swiss albino mice. T'6 tumor was transplanted successfully into two of five btk mice. In Swiss albino mice however, only one of five was transplanted nineteen generations. (Table 3)

Table 3. Trsnsplantation of the tumor in various strains of mice.

Strain of tumor	Strain of mouse		Route ** of transplantation	Generation of transplantation	Number of mouse transplanted				
	Tumor produced	Tumor transplanted			Total	Valid	+	−	Rate (%)
T'6	ctk	Swiss albino	sc	1	5	5	1***	4	20
		ddO	〃	1	5	5	0	5	0
		btk	〃	1	5	5	2	3	40.0
		C57BL	〃	1	5	5	0	5	0
		C3H	〃	1	5	5	0	5	0
		ctk	〃	1	5	5	5	0	100.0
		〃	ip	1	4	4	4****	0	100.0

* In this experiment a tumor T'6 was used which was obtained in the experiments reported previously (Kawamata *et al.*, 1958).

** sc: Subcutaneous transplantation of the tumor.
ip: Intraperitoneal transplantation of the tumor.

*** This transplanted tumor was transplanted to Swiss albino mice for 19 generations. Rate of successful transplantation was 76.1 %.

**** Until the present time this tumor has been transplanted into ctk mice for 12 generations. Rate of successful transplantation was 96.1 %.

Histologically these transplanted tumors closely resemble the primary tumors. (Fig. 10)

Metastasis of the primary tumors was not observed. However, after several intraperitoneal transplantations, some mice developed a large number of small metastatic tumors in the peritoneum, omentum, serosa of the intestine and abdominal lymphatic nodules. Histologically they were identical with transplanted tumors. (Fig. 11, Fig. 12)

In one series of transplantations, mice bearing ascites tumor were observed. Fig. 13 shows the ascites cells stained by Giemsa solution. Details of this ascites tumor will be reported in the next paper.

DISCUSSION

In the previous paper it was reported that a sarcoma was produced in mice after long term administration of actinomycin S. In this paper it was shown that not only actinomycin S but also actinomycin L can produce sarcomas in mice. Though actinomycin S induces sarcomas in btk and ctk mice at a high rate, in other stocks and strains of mice such as Swiss albino, ddO and C57BL/De, sarcoma formation was much less. No sign of tumor formation was observed in C3H/MsMa mice, but since a large number of mice died during the experiment, the results on this strain may not be conclusive.

The amount of the drug administered greatly influences tumor production.

Though the carcinostatic activity of actinamycin L is about one third of that of actinomycin S, carcinogenic activity of the former is more than one tenth of the latter. It is conceivable that the ratio of carcinostatic to carcinogenic activity is not always be equal for different drugs. But the relation shown in actinomycin S and L suggests that a drug which has lesser carcinogenic activity than another drug, may nevertheless have the same carcinostatic activity.

An ideal carcinostatic drug should have no toxicity to the host and no carcinogenicity. Most carcinostatic drugs however, are thought to produce tumors. Therefore, a drug which is worth testing clinically, should have a big difference between carcinostatic and carcinogenic activities. In this respect, actinomycin L is better than actinomycin S. But to test its effectiveness further experiments will be required.

Why actinomycin produces sarcomas in mice is not clear. But as reported by Allen *et al.* (1957), 3-hydroxyanthranilic acid and 3-hydroxykynurenine produce cancer of the bladder in mice. It is not yet known whether 3-hydroxyanthranilic acid and 3-hydroxykynurenine act *per se* as carcinogens or whether their condensation products such as phenoxazone derivatives, might play a role. If this is so, the phenoxazone moiety of actinomycin may play an important role in carcinogenesis. Also, as postulated by Bergel (1958), the peptide chains of actinomycin might serve as "carriers" for the biological activity of the phenoxazone. Differences in carcinogenic activity of actinomycins may be attributable to differences in their peptide chains.

Since natural substances such as actinomycins cause a high incidence of sarcomas in mice and the chemical structure resembles that of tryptophan metabolites, there might be an endogenous carcinogen which causes tumors in man and animals.

The biochemical mechanism of the action of actinomycin on living organisms has been investigated in our laboratory (Sayanagi, 1959; Imanishi, 1959), and by Foley (1956) and Slotnick (1956, 1958). Results show that actinomycin inhibits protein synthesis in microorganisms. Moreover we have found that actinomycin combines with deoxyribonucleic acid of bacteria and calf thymus (Imanishi, 1959; Kawamata *et al.*, 1959).

Further investigation of the mechanism of the action of actinomycin is now in progress.

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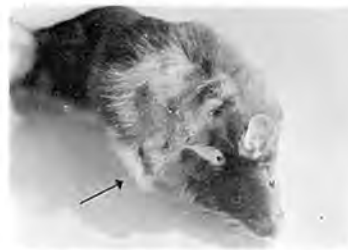

Fig. 1. Advanced sarcoma in btk mouse at 163 days. Arrow indicates the localization of tumor.

Fig. 2. Torso of mouse of Fig. 1 opened to show lesion. Arrow indicates tumor.

Fig. 3. Excised tumor from the same mouse as in Fig. 1.

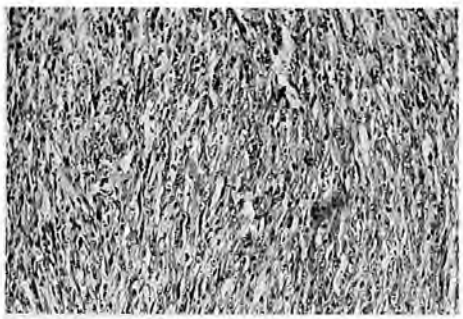

Fig. 4. Photomicrograph of sarcoma produced in btk mouse at 122 days. H. and E. stain ×73

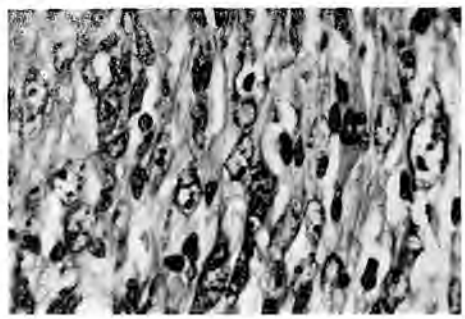

Fig. 5. Photomicrograph of sarcoma produced in btk mouse at 122 days. H. and E. stain ×310

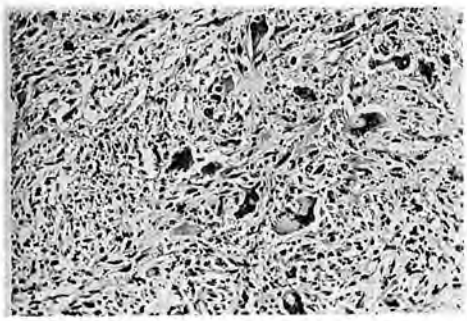

Fig. 6. Photomicrograph of sarcoma produced in btk mouse at 166 days showing giant cells. H. and E. stain ×73

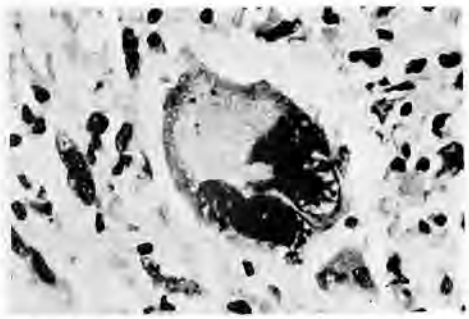

Fig. 7. Photomicrograph of sarcoma produced in btk mouse at 166 days showing giant cells. H. and E. stain ×310

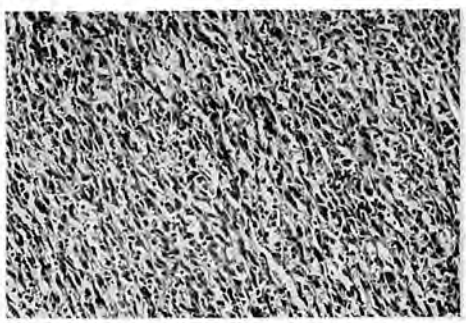

Fig. 8. Photomicrograph of sarcoma produced in Swiss albino mouse at 192 days. H. and E. stain ×73

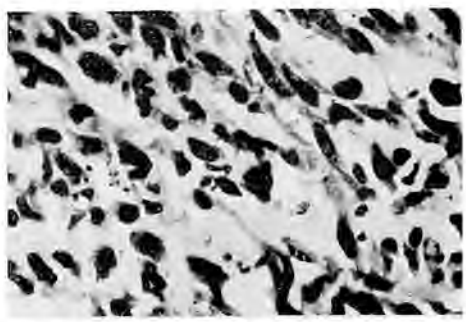

Fig. 9. Photomicrograph of sarcoma produced in Swiss albino mouse at 192 days. H. and E. stain ×310

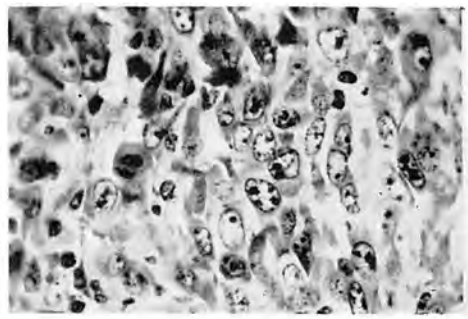

Fig. 10. Photomicrograph of 11th generation transplant in ctk of tumor from ctk at 18 days. H. and E. stain ×310

Fig. 11. Metastasis of 11th generation transplant in ctk of tumor from ctk at 18 days. Arrow indicates the localization of metastasis.

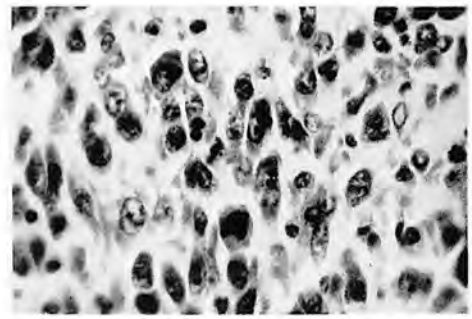

Fig. 12. Photomicrograph of Fig. 11. H. and E. stain ×310

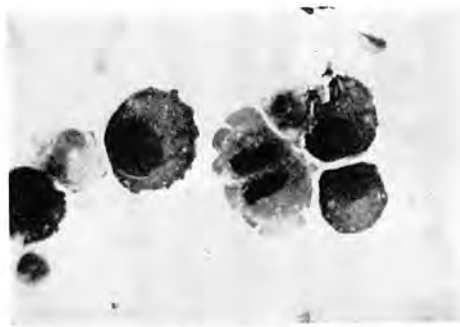

Fig. 13. Photomicrograph of tumor cells in ascites of 2nd generation transplant at 7 days. Giemsa stain ×310