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Effect of Estramustine Phosphate (Estracyt®) on Transplantable Mouse Tumours

M. Wakisaka[1], I. Iwasaki[2] and J. Shimazaki[1]

[1]Department of Urology and [2]Department of Pathology, School of Medicine, Chiba University, Chiba, Japan

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Summary. Treatment of mice bearing levulose sarcoma with estramustine phosphate showed a cytotoxic effect which was similar to that of cyclophosphamide. Therefore, the alkylating moiety of estramustine phosphate seems to be the main principle of the cytotoxic effect observed. However, this effect of estramustine phosphate was observed only on the levulose sarcoma among the mouse tumours examined. Estramustine phosphate also inhibited the growth of androgen dependent and independent mouse mammary tumours (SC-115 and SC-115-Independent). These effects of estramustine phosphate were also evoked by injection of estradiol-17β, 3, 17-diphosphate, therefore it seems to be due mainly to the oestrogenic activity of the compound.

Key words: Estramustine phosphate - Levulose sarcoma - Androgen dependent and independent tumours.

Recently, estramustine phosphate[1] has been used for control of reactivated prostatic cancer and the clinical usefulness of this compound is generally accepted (2, 10, 14, 18, 19). It was found that administration of estramustine phosphate evoked involutional changes in the prostates of rats, dogs (12) and baboons (20). DNA synthesis of the rodent prostate was inhibited by estramustine phosphate (6, 9). However, the mechanism of the action of this compound is still disputed, because estramustine phosphate possesses both alkylating and the oestrogen moieties in the molecule. To clarify further the action of estramustine phosphate on tumour growth, we examined the effect of estramustine phosphate on some transplantable mouse tumours.

[1]Estramustine phosphate (Estracyt[R], estra-1, 3, 5 (10)-triene-3, 17β-diol, 3-N-(bis(2-chloro-ethyl)) -carbamate-17-dihydrogen phosphate) was supplied by Leo, Helsingborg, Sweden

MATERIALS AND METHODS

Animals

Male mice of DD/S strain weighing approximately 25 g were used. Animals of this strain were originally derived from the Shionogi Laboratory, Japan, and have been bred in our laboratory.

Transplantable Mouse Tumours

Tumours used in the present experiments were as follows: (A) levulose sarcoma (442nd-455th generation) which was developed and maintained at the Department of Pathology, School of Medicine, Chiba University (24), (B) SC-42 (319th-326th generation), androgen independent mammary tumour which was spontaneously developed (1959) and maintained at the Shionogi Laboratory (16), (C) SC-115 (169th-175th generation), androgen dependent mammary tumour which was spontaneously developed (1961) and maintained at the Shionogi Laboratory (1, 11, 16, 17, 25), (D) SC-115-Independent (258th-269th generation), the androgen dependency of this tumour had mostly disappeared during passages of SC-115 in our laboratory. At this time, transplantable ratio in animals of both sexes was 100%.

Implantation of Tumours and Treatment of Animals

A piece of tumour, approximately 5 mg in wet weight (approximately 10^7 cells), was implanted

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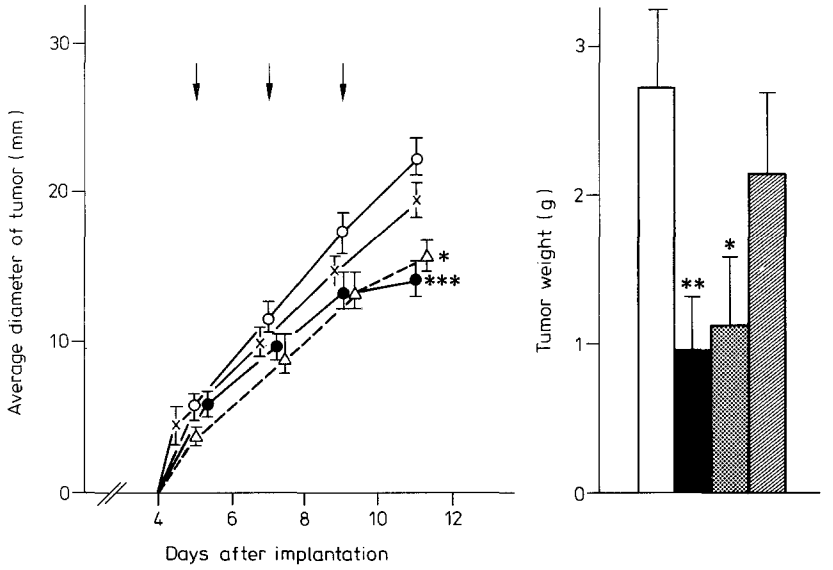

Fig. 1 Growth of levulose sarcoma: Average tumour diameter (left) and tumour weight at 48 hrs after the final injection (right) were shown as M ± SE. A dose of 0.1 ml of destilled water (open circle in left, open bar in right), or 40 mg/kg of cyclophosphamide (closed circle in left, closed bar in right), or 76 mg/kg of estramustine phosphate (open triangle in left, dotted bar in right) or 64 mg/kg of oestradiol diphosphate (cross in left, hatched bar in right) were injected mice on alternate days (arrows). Numbers of animals used in this experiment was 20, 20, 16 and 16 for controls, cyclophosphamide-treated, estramustine phosphate-treated and oestradiol diphosphate-treated groups, respectively. Statistical differences: *($p < 0.05$), **($p < 0.01$), ***($p < 0.001$)

subcutaneously with a trocar in the dorsal midline of the neck of recipient mice. After the tumour had grown to be palpable as a small mass, the animals were divided into four groups and the following intraperitoneal injections were performed on alternate days: group 1 - 0.1 ml of distilled water; group 2 - 40 mg/kg of cyclophosphamide in 0.1 ml; group 3 - 76 mg/kg of estramustine phosphate in 0.1 ml and group 4 - 64 mg/kg pf oestradiol diphosphate (oestradiol-17β, 3,17-diphosphate) in 0.1 ml. Animals were sacrificed 48 hrs after the final injection. Tumours were excised, weighed, then processed for histological examination.

RESULTS

Effects on Levulose Sarcoma

This tumour usually became palpable on the 5th day after implantation, then tumour size increased gradually (Fig. 1, left). Treatment with cyclophosphamide and estramustine phosphate evoked a significant decrease in tumour growth, however, oestradiol diphosphate injection did not show any influence on the growth of tumour. The tumour weight at the time of sacrifice is shown in Fig. 1, right.

In the control group (group 1), tumour cells were pleomorphic and the nuclei were round to oval with distinct nucleoli (Fig. 5). The pleomorphism of tumour cells became more prominent after treatment with cyclophosphamide, and sometimes polygonal giant cells were found. There were also some ghost cells and degenerative cells with pyknotic nuclei (Fig. 6). A similar degenerative pattern was observed in tumours treated with estramustine phosphate, but the degree of change was less in this group than in the cyclophosphamide treated group (Fig. 7). No histological changes were seen in the tumour cells treated with oestradiol diphosphate.

Effects on SC-42 Tumours

Most of these tumours were palpable on the 8th day following implantation, and then the tumour showed gradual growth with time (Fig. 2, left). Administration of cyclophosphamide, estramustine phosphate or oestradiol diphosphate did not influence the tumour growth markedly (Fig. 2, right). However, tumour weight at the time of sacrifice was less in cyclophosphamide injected mice than in the other three groups.

There was no histological difference between the tumour cells of the four experimental groups.

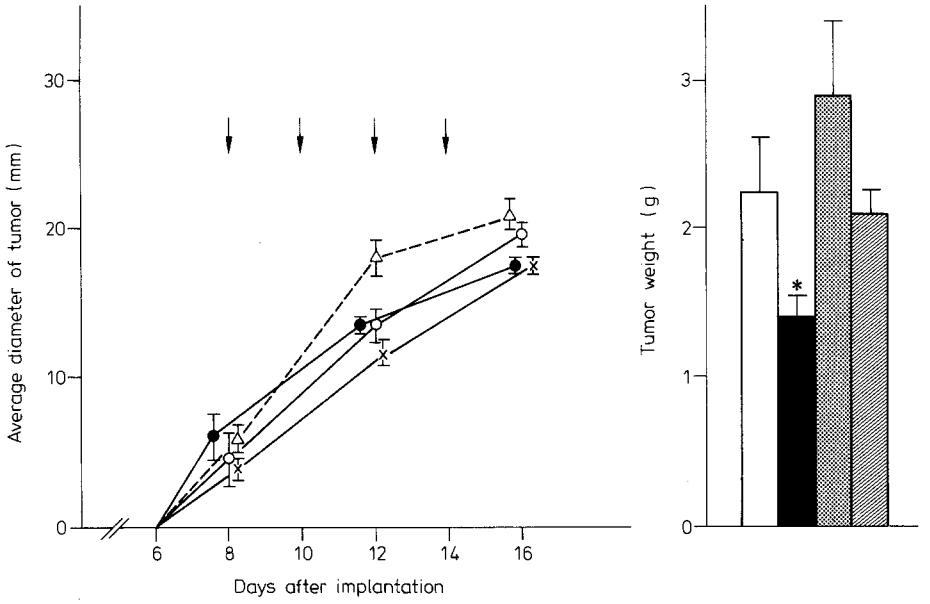

Fig. 2. Growth of SC-42: Treatments and symbols are the same as those in Fig. 1. Numbers of animals used in this experiment were 15, 16, 17 and 16, respectively.

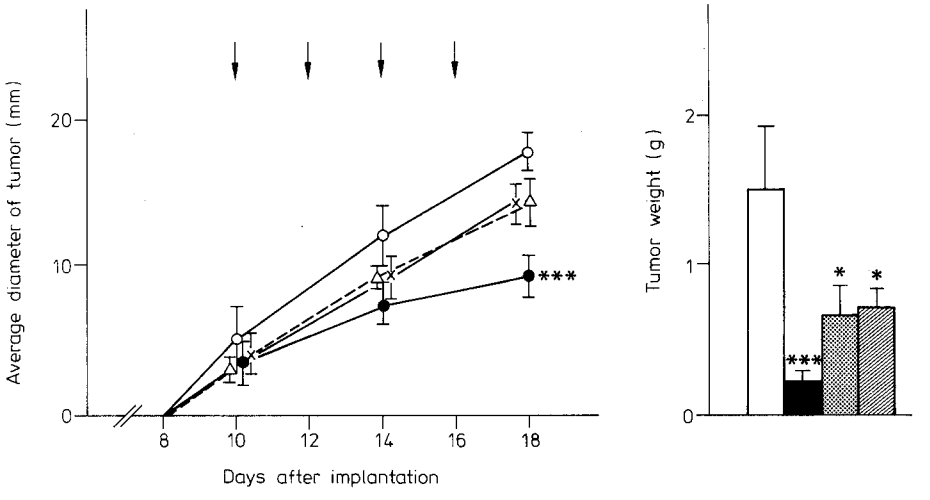

Fig. 3. Growth of SC-115: Treatments and symbols are the same as those in Fig. 1. Numbers of animals used in this experiment were 19, 16, 21 and 17, respectively.

Effects on SC-115 Tumours

Growth rate of SC-115 tumour was the slowest among the tumours used in the present study. The tumour was usually palpable on the 10th day after implantation, then grew gradually (Fig. 3, left). Growth rate of the tumour was significantly inhibited by treatment with cyclophosphamide.

Moderate inhibition of the tumour was also evoked by estramustine phosphate or oestradiol diphosphate. The tumour weight at the time of sacrifice was significantly reduced in all of these three groups (Fig. 3, right).

Histological examination of tumours of group 1 revealed that the tumour consisted of round cells and a large population of cells which showed an epithelial growth pattern forming glandular-like structures, occasionally with complete lumen. Nuclear membranes were hyperchromatic and nucleoli were also distinct. This tumour contained many necrotic foci (Fig. 8). In the cyclophosphamide treated group, much cellular pleomorphism was found and the cytoplasm of most of these cells contained fine vacuoles. Cell necrosis and abnormal mitoses were observed in moderate numbers. Cellular degeneration was noticed in

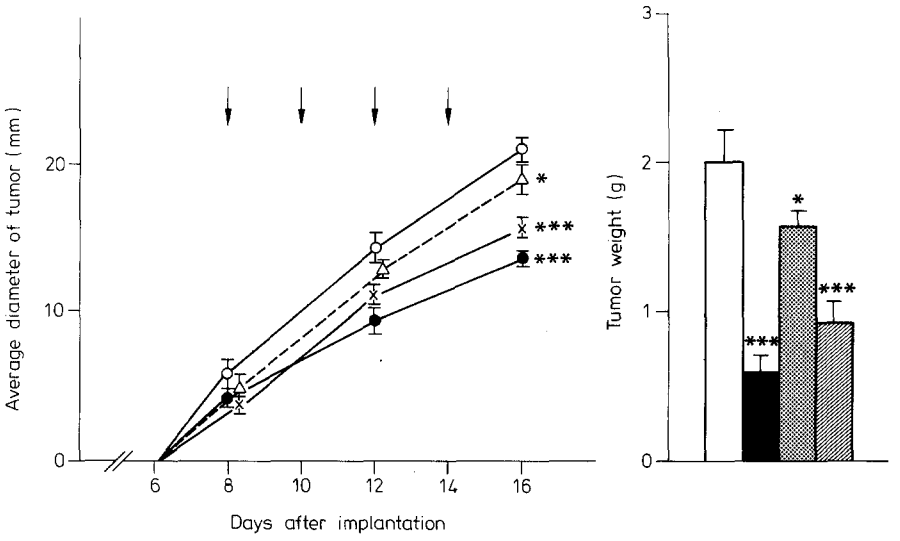

Fig. 4. Growth of SC-115-Independent: Treatments and symbols are the same as those in Fig. 1. Numbers of animals used in this experiment were 15. 16, 15 and 17, respectively.

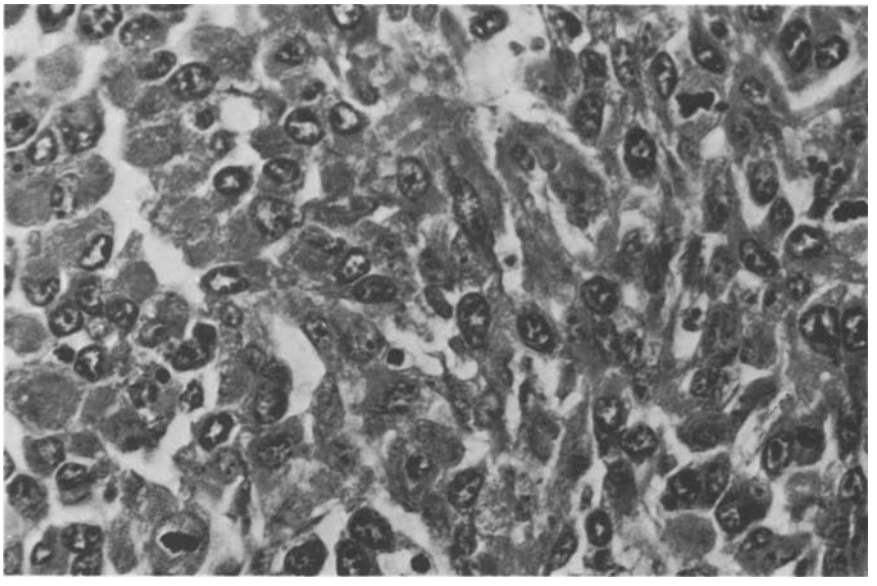

Fig. 5. A section of control group, showing pleomorphic sarcoma with many mitoses (levulose sarcoma, H & E, X 400)

some areas of the tumour (Fig. 9). In the estramustine phosphate treated group, moderate degrees of the cellular pleomorphism with abnormal mitoses were observed and the nuclear membrane was obscure in many cells. These changes seem to be quite different from those observed in tumours from the cyclophosphamide treated group (Fig. 10). Oestradiol diphosphate evoked similar degenerative changes to those observed in the estramustine phosphate treated mice.

Effects on SC-115-Independent Tumours

This tumour grew faster than the SC-115 tumour and the tumour was palpable approximately two days earlier (on the 8th day) than the dependent tumour. Growth rate of the tumour was inhibited significantly by treatment with either cyclophosphamide, estramustine phosphate, or oestradiol diphosphate (Fig. 4, left). Tumour weight was measured on the 16th day after implantation, and significant reductions were noticed in all of these three groups (Fig. 4, right).

Histologically, the tumour tissue in control animals (group I) showed almost same appearance as the equivalent SC-115 tumour. In the cyclophosphamide treated group, cellular polymorphisms and degenerative changes were evident. Giant cells varied in size and the shape. Occasionally abnormal mitoses were seen. Estramustine phosphate treatment evoked a cel-

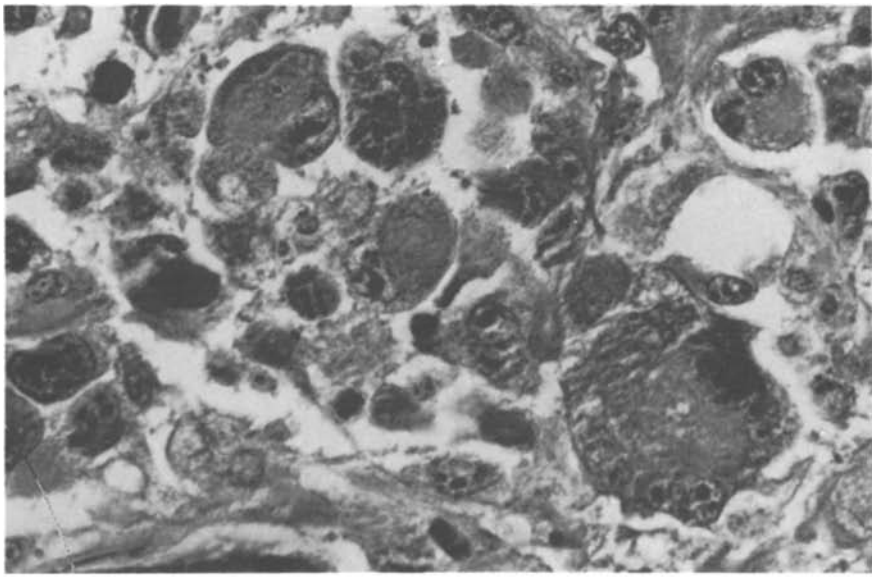

Fig. 6. A section of cyclophosphamide-treated group, showing marked polygonal or polynuclear giant cells and cell necrosis (levulose sarcoma. H & E, X 400)

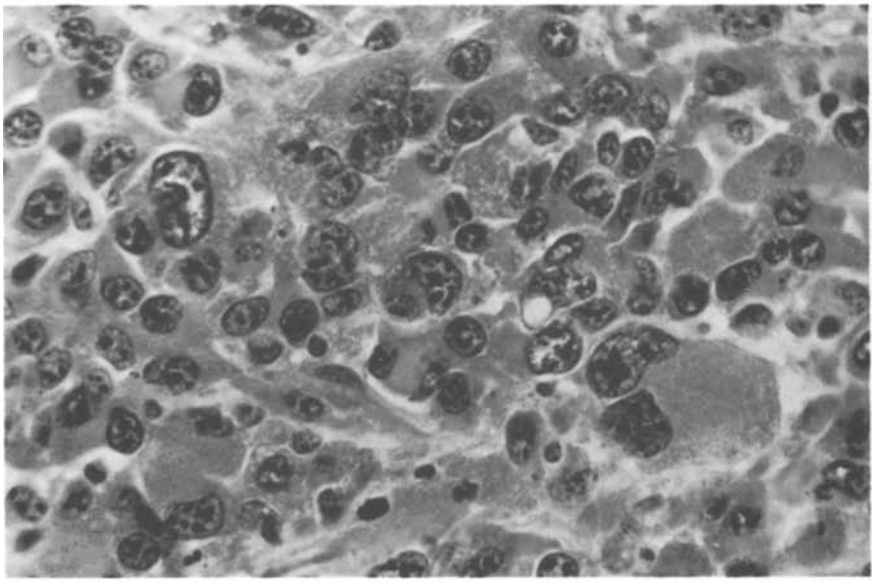

Fig. 7. A section of estracyt-treated group, showing a moderate cellular pleomorphism and a few polynuclear giant cells (levulose sarcoma, H & E, X 400)

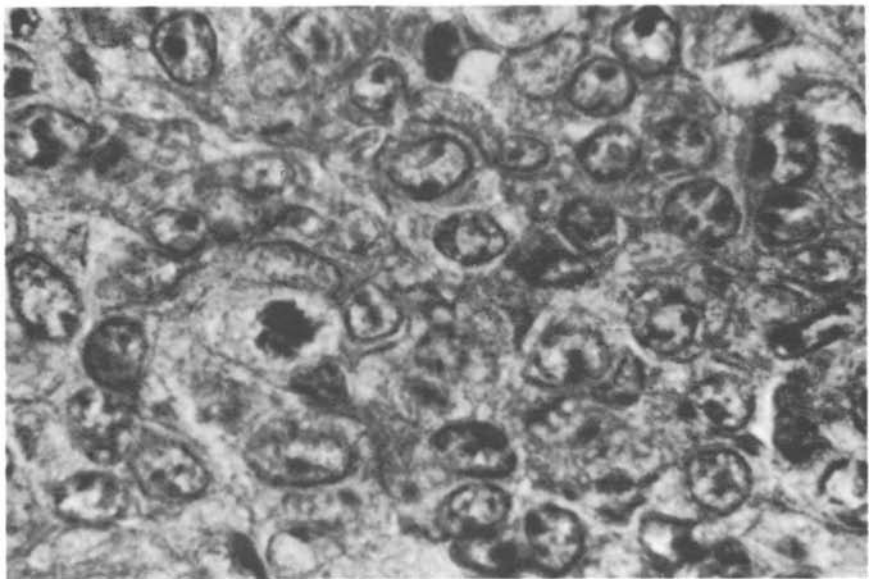

Fig. 8. A section of control group, showing medullary carcinoma with hyperchromatic nuclear membrane and prominent nucleoli (SC-115, H & E, X 800)

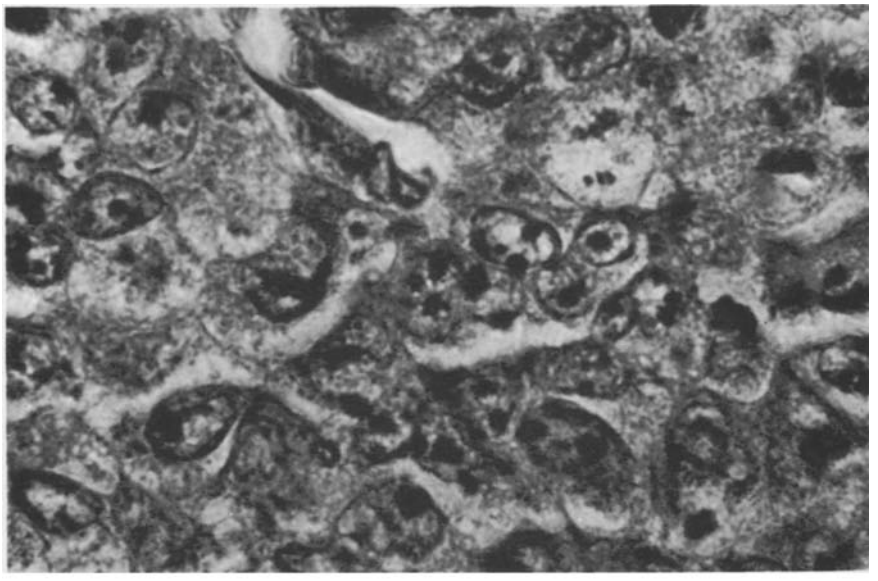

Fig. 9. A section of cyclophosphamide-treated group, showing moderate pleomorphism and cell necrosis with abnormal mitoses (SC-115. H & E, X 800)

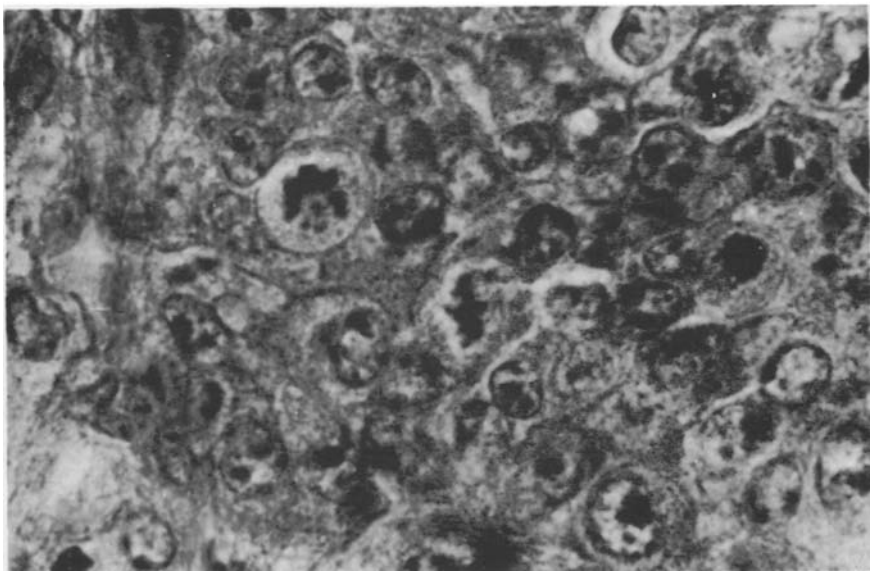

Fig. 10. A section of estracyt-treated group, showing obscure nuclear membrane with moderate cellular pleomorphism and abnormal mitoses which are apparently different from those of cyclophosphamide-treated group (SC-115. H & E, X 800)

lular degeneration and pleomorphism as well as pyknosis to a slight degree, and these changes were similar to those observed in the SC-115 tumour after estramustine phosphate treatment. Similar degenerative changes of the tumour were induced in less degree by oestradiol diphosphate treatment. Although SC-115-Independent tumour grew in female mice, the growth rate of the tumour was slower in female mice than in male animals. Therefore, administration of a large dose of oestrogen used in the present experiments might suppress the growth of this tumour.

DISCUSSION

It was observed in our laboratory that the growth rate of levulose sarcoma was almost identical in male and female mice. In the present experiments, no significant changes in tumour growth were observed in the oestrogen-treated group. However, treatment with estramustine phosphate showed a cytotoxic effect which resembled that observed in the cyclophosphamide - treated mice (23). It has been reported by Fredholm et al. (4) that estramustine phosphate showed an antitumour activity which was different from oestrogenic activity to a DMBA-induced mammary tumour of rats. In the present experiments an antitumour effect of estramustine phosphate which completely differed from oestrogenic activity was observed only in levulose sarcoma. This might be due to high susceptibility of levulose sarcoma to the alkylating moiety of estramustine phosphate which might act either in the bound form with oestrogen or in the free form which had split off from the

molecule in vivo. Actually, it has been proved that estramustine phosphate is metabolised to form dephosphorylated compounds first and then further converted to free steroid and carbamate moiety in vivo and also in vitro (3, 8, 13)

Suppression of growth rate of SC-115 and SC-115-Independent tumour in estramustine phosphate-treated mice observed in the present experiments seems to be mainly attributable to the oestrogenic activity of estramustine phosphate, since oestradiol diphosphate also showed some anti-tumour effects. Although the rate of inhibition on prostatic testosterone 5 α-reductase activity and androgen receptor complex formation by estramustine phosphate was lower than that exerted by oestrogen (5, 7), it has been reported that the effect of estramustine phosphate on the prostate was mainly due to its oestrogenic potency (26). According to McMillin et al. (15), the effect of estramustine phosphate on pituitary, gonadal and adrenal function also appeared to be exerted by its oestrogenic activity.

Recently, Müntzing et al. (21) reported an antitumour effect of estramustine phosphate on a transplantable prostatic cancer of rats (R-3327). It was also documented that the inhibitory effect on growth of the same cancer by estramustine phosphate was higher than that by diethylstilboestrol (22). These results suggest that the antitumour effect of estramustine phosphate on this tumour is not accounted for only by the oestrogenic activity of the molecule. However, it is not possible from this kind of experiment to distinguish whether or not estramustine phosphate acts as an oestrogen or an alkylating agent alone or in combination. Although in the present experiments a specific cytotoxic effect of estramustine phosphate on the growth of SC-115 tumour was not observed, further study may elucidate the mechanism of effects of estramustine phosphate on androgen dependent tumours.

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Dr. M. Wakisaka
Department of Urology
School of Medicine
Chiba University
Chiba
Japan

Responsible for the text: Professor G. D. Chisholm, Western General Hospital, Edinburgh.
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