

Leiomyosarcoma occurring in CRJ-SD Male Rat fed 20% Casein Diet for Long Period.

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Abstract

A tumor was found in one of the rats (Charles-River CRJ-SD strain) fed 20% casein diet for long period. The detail of growth, symptom and histochemical findings were presented here.

For about 43 weeks the rat grew well showing 585 g of body weight. But thereafter it decreased gradually and diarrheas were sometimes observed with severe weight loss, nearly 100 g of loss in the last 17 experimental days. The tumor was found in the right-side region of retroperitoneum. Its dimensions were $30 \times 20 \times 15$ mm. It was sharply circumscribed and fixed to the adjacent muscular tissues.

Microscopically, there were elongated spindle-shaped tumor cells with large blunt-ended nuclei which were hyperchromatic and partly vesicular. Tumor cells were very pleomorphic and mitosis was also observed. The degeneration and necrosis were found in the central portion of the tumor mass, and otherwise, the tumor cells infiltrated into the adjacent muscular tissues.

From these results, it was suggested that the tumor would be a leiomyosarcoma arising from retroperitoneum in a male rat.

Key words: leiomyosarcoma, male rat (CRJ-SD strain), 20% casein diet, retroperitoneum.

Introduction

Leiomyosarcoma which is one of the malignant tumors in the somatic soft tissues, is relatively uncommon in the human beings. According to the Anderson's pathology (1), they account for 2 to 8% of soft tissue sarcomas and are found in the superficial subcutaneous tissues and skin. In deep soft tissues the retroperitoneum is the most frequent site with other sites including the mesentery, the omentum, and major blood vessels. The retroperitoneal lesions and those arising from major blood vessels are more common in female subjects (1,2,3,4).

From literature (5) or data-book of Charles-River CRJ-SD strain rats (6), leiomyosarcoma in the rat somatic soft tissues did not occur in male rat but a few in female.

We could observe a case of the leiomyosarcoma in a male rat fed 20% casein diet for long period. Therefore, detail of this leiomyosarcoma occurred in a male rat is presented here.

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Materials and methods

Diet and animals

Diet: The experimental diet was 20% casein diet consisted of vitamin free casein (Oriental Yeast Co., Ltd.¹) at a 20% level, 5% of corn oil (Ajinomoto Corp. Inc.²) and 68.8% of corn starch (Nichiden-kagaku Corp., Ltd.³). This diet also contained considerable amounts of vitamins (1% of vitamin mixture according to Harper (7)), choline chloride (0.2%) and 5% of salt mixture.

Animals: The five male rats of Charles-River CRJ-SD strain (Nihon Charles-River Inc.⁴), at 4 weeks of age, weighing 75 to 85 g were used in the experiment. They were individually housed in wire cages in a room which was automatically light controlled to provide a 12 hours darkness and light cycle, at $22 \pm 1^\circ\text{C}$, at 60% of the relative humidity. The rats were given standard rat pellets (CE-2, produced by Nihon Clea Inc.⁵) for 7 days before the experiment. The rats were maintained on the 20% casein diet. They had free access to the diet and tap water. Food intake and body weight for each animal were measured at the same time of every other day.

Autopsy: Autopsy was carried out immediately after death and small pieces of organ and tissue specimens were fixed with 10% formaldehyde solution. After sufficient fixation, tissue blocks cut off from the organ and tissue specimens were dehydrated for about 6 hours and embedded into paraffin by using of the Handex autokinette (model 900, Shiraimatsu Corp. Ltd.⁶). Paraffin sections (3 to 5 μ thick) were stained by hematoxylin-eosin stain, van Gieson's method and Mallory-azan method (8,9,10).

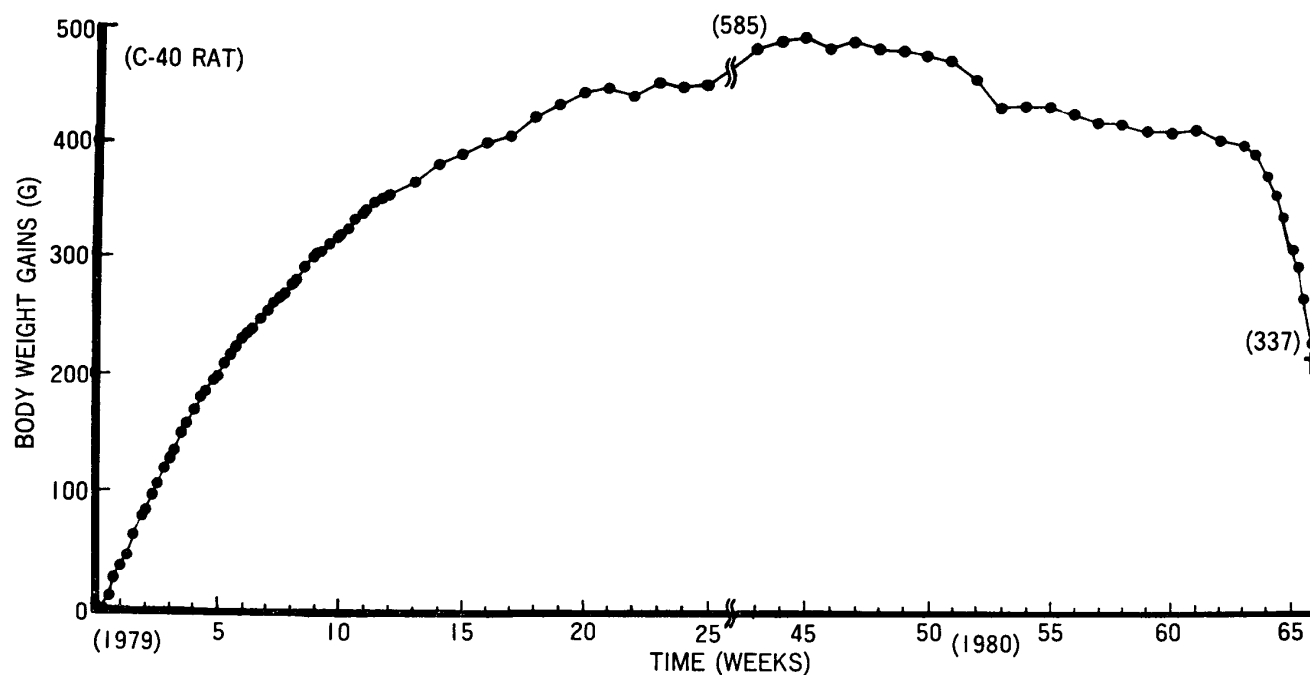


Fig. 1. Growth curve of the rat fed 20% casein diet for long period.

- 1) Oriental Yeast Co., Ltd. (3-6-10, Azukizawa, Itabashi-ku, Tokyo 174, Japan)
- 2) Ajinomoto Corp. Inc. (1-6, Kyobashi, Chuo-ku, Tokyo 104, Japan).
- 3) Nichiden-Kagaku Corp., Ltd. (3-29, Mitsuyakita-Sanchome, Yodogawa-ku, Osaka 532, Japan).
- 4) Nihon Charles-River Inc. (795, Shimofurusawa, Atsugi, Kanagawa Pref. 243-02, Japan).
- 5) Nihon Clea Inc. (4-234, Otsuka-cho, Takatsuki, Osaka 569, Japan).
- 6) Shiraimatsu Corp., Ltd. (19-16, Chuo-1-chome, Morinomiya, Higashi-ku, Osaka 540, Japan).



Photo. 1. Localization and gross feature of the tumor. Arrow shows the tumor. This photograph was taken after formaldehyde fixation. Two parts of the tumor were cut off for histological determination.

Results and discussion

Growth and symptom

As shown in Fig. 1, the body weight of the rat fed 20% casein diet for 43 weeks was 585 g of the almost maximum. Thereafter, it gradually decreased and was 531 g at the 57 weeks, and diarrheas were sometimes observed with severe weight loss, nearly 100 g of loss in the last 17 experimental days. The rat died at the 66 th experimental week. The body weight of the rat at the death was 337 g. When autopsy was carried out immediately, a tumor was found in the right-side region of retroperitoneum as shown in Photo. 1.

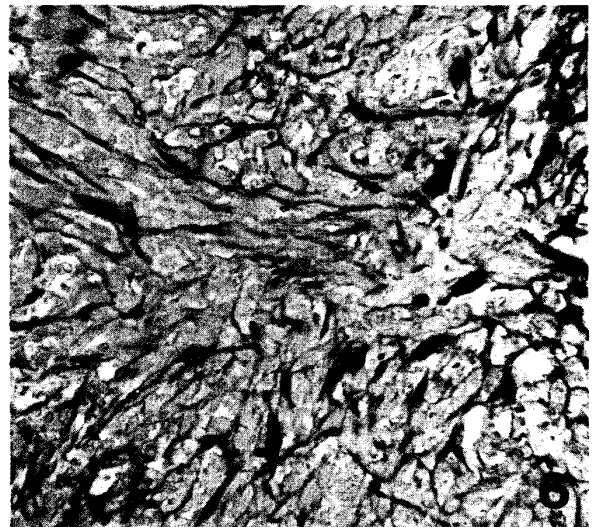
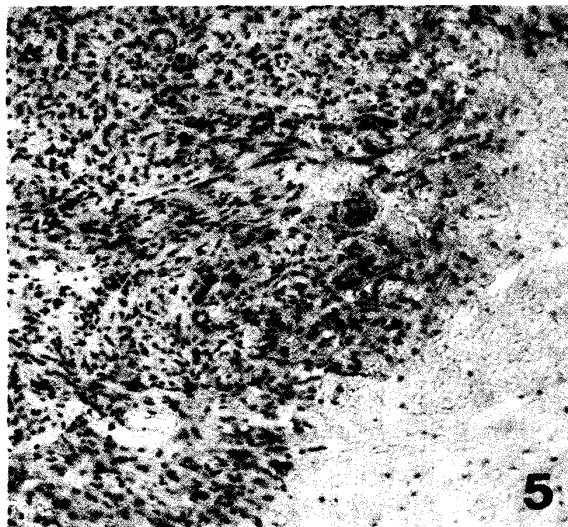
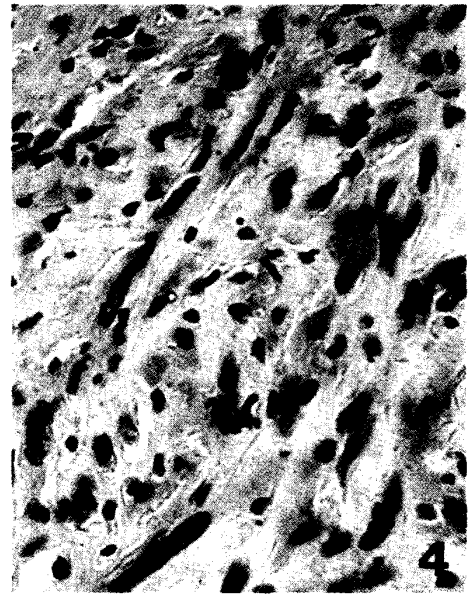
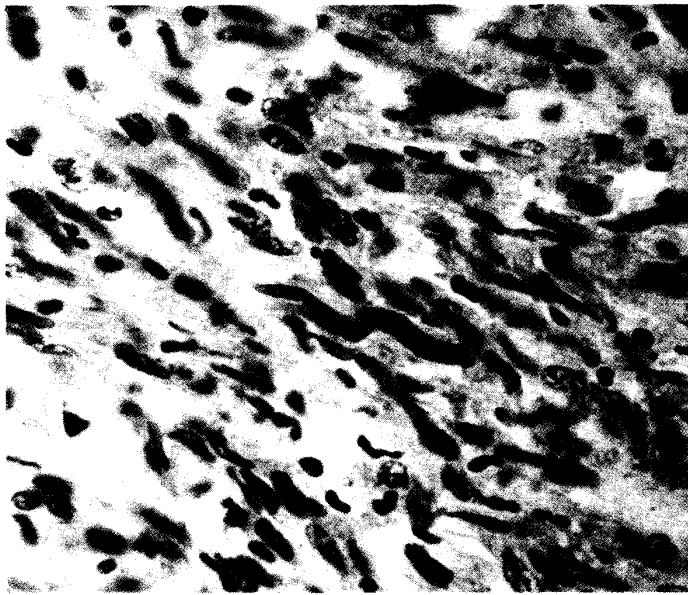
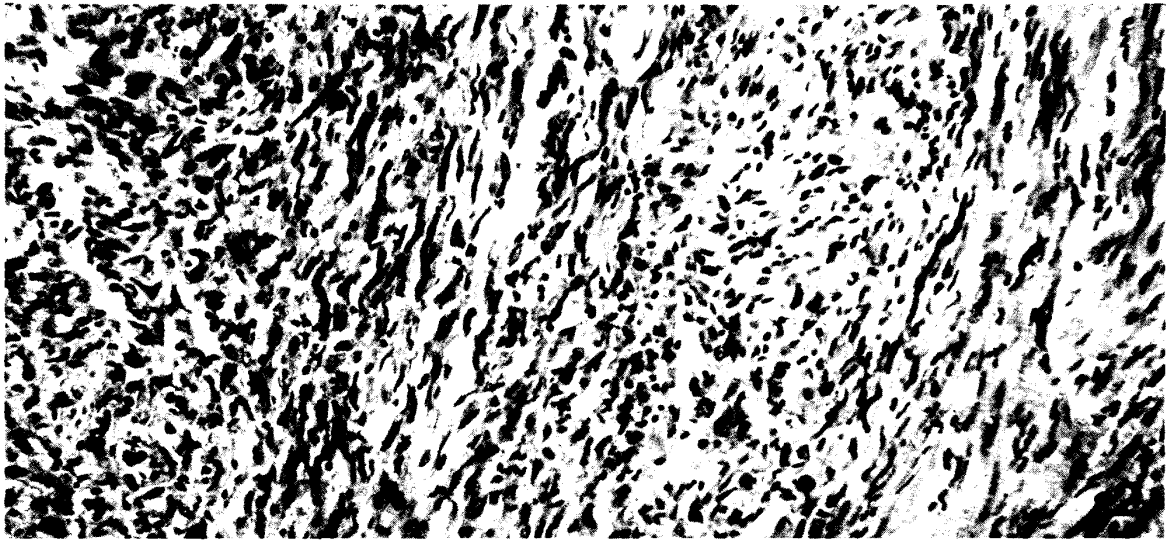
Macroscopical finding of the tumor.

Its dimensions were $30 \times 20 \times 15$ mm. It was sharply circumscribed and fixed to the adjacent muscular tissues. The tumor was firm and gray in colour.

Microscopical finding of the tumor.

As shown in Photo. 2 (low magnitude), bundles of spindle-shaped cells are seen in the histological section of the tumor. The bundles of the tumor cells are seen partly in longitudinal (right and left sides of the photo. 2) and cross section (middle of the photo.). The hemorrhagic, degenerative and necrotic features are found in the central area of the tumor mass (right side in photo. 5). As shown in Photo. 6, by van Gieson's stain, the tumor cells are yellowish in colour and on the other hand, connective network consisting of fine collagenous fibers is reddish. In Photo. 3 and 4 (high magnitude), the tumor cells are in spindle-shaped or oval form with large blunt-ended nuclei which are hyperchromatic and partly vesicular. The varieties in size and appearance of the tumor cells are observed. Thus tumor cells are very pleomorphic. The mitosis was also observed.

As described by Greaves and Faccini (5), reports of leiomyosarcoma in the rat are quite a few.



- Photo. 2. Histological section (hematoxylin-eosin stain) of the tumor (low magnitude, $200\times$).
Bundles of smooth muscle fibers are seen partly in longitudinal and cross section.
- Photo. 3. & 4. Histological sections (hematoxylin-eosin stain) of the tumor (high magnitude, $400\times$).
Elongated spindle-shaped tumor cells with blunt-ended nuclei are seen.
- Photo. 5. Central region of the tumor mass.
Hemorrhagic, degenerative and necrotic features are seen in right side of photograph.
- Photo. 6. Histological section (van Gieson's stain) of the tumor ($200\times$).

According to data-book of Charles-River Inc., spontaneous leiomyosarcoma arise in 0/200 of the incidence for male rat and in 1/200 for female in 90 to 104 weeks survival period. Thus it is probably considered that leiomyosarcoma in the rat may be a few. In this case, the tumor arising from retroperitoneum of a male rat was relatively large and circumscribed. As shown in the photographs, tumor cells are very pleomorphic and mitotic activity was observed. Otherwise, the tumor cells which was stained yellow by van Gieson's method, would be muscle fibers. The hemorrhagic, degenerative and necrotic features were found in the central area of the tumor mass.

As pointed out in Anderson's pathology (1), it is difficult to differentially diagnose between two tumors arising from smooth muscle (leiomyoma and leiomyosarcoma). According to Thomas (11), the leiomyosarcoma cells which are very pleomorphic have mitotic activity, and Kissane (1) pointed out that necrosis in the tumor mass is important finding in differentiating benign from malignant tumor.

From these consideration and our results, it was suggested that the tumor would be leiomyosarcoma arising from retroperitoneum in a male CRJ-SD rat.

Furthermore, by Greaves and Faccini's suggestion (5) that ultrastructural study is helpful for diagnosis of smooth muscle tumor, electron-microscopical determination of this tumor will be performed.

At present, we have no explanation for the problem of relationship between the genesis of this tumor and nutrition. Thus we considered for this tumor to occur spontaneously.

Acknowledgement

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20%カゼイン飼料長期投与 CRJ-SD 雄ラットに 発症した平滑筋肉腫について

要 旨

20%カゼイン飼料長期投与 CRJ-SD 雄ラットの1例に腫瘍が発症した。このラットの体重変動、症状及び剖検時ならびに組織学的所見を報告する。

43週まで体重 585 g を示して順調に成長していたラットがその後次第に体重減少を来し、死亡前17日間で体重が約 100 g も減少し、それにともなって下痢症状が現われた。死後剖検時に右側後腹壁に 30×20×15 mm の腫瘤を認めた。この腫瘤は限局性で底部は周囲組織に固着していた。

顕微鏡観察で大きな blunt-ended な核をもつ紡錘形の腫瘍細胞がみられ、核は hyperchromatic な部分と vesicular な部分とが見られ、細胞の異型性が強かった。また分裂像も認められた。一方腫瘤中心部には変性、壊死像も観察された。

以上の所見からこの腫瘍は後腹膜壁に発症した平滑筋肉腫と考えられる。