cleavage of a 3'-OH terminal fragment of 49 nucleotides from rRNA-molecules[10], in spite of the presence of both 18S- and 16S-rRNA. It also probably does not directly hit cellular DNA-molecules. Its effect may be characterized as cytotoxic rather than cytostatic.

In sensitive bacteria, colicin E3 - similarly as other ones - developes its effect in 2 phases: the first, bacteriostatic one (started immediately through adsorption on plasma membrane receptor), is succeeded in about 30 min by the second, bactericidal one[11]. It is well possible that in leukemia cells P388, the effect of E3 is limited to an analogy of the first one.

In this context, the substantially lower killing of P388 cells than of HeLa- and L-cells by colicin E3 is worthy of notice: while about 1×10^4 l.u. per cell are sufficient to kill 100% of cells HeLa, about 3×10^5 l.u. per cell for killing 100% of cells L during 3 days[5] and about 7.4×10^5 l.u. per cell for killing 29% cells of a human mammary carcinoma MDA 231 MB during 1 day[6], about 4.4×10^5 l.u. per cell suffice to kill only 1.7% of leukemia P388 cells in 3 days, although under different experimental conditions. This difference of 2 ten-orders is related to similar sensitivity differences found for most known cytostatics.

Preliminarily, pure colicin E3 appears to be nontoxic for laboratory animals and its pyrogenicity is very low. Thus it seems plausible to analyse its effect on tumor cells, especially on cells of other tumor types, in greater detail.

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A histochemical study of denervated marsupial hemidiaphragm

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Summary. The marsupial (quokka) hemidiaphragm showed postdenervation hypertrophy and subsequent atrophy. The type II muscle fibres hypertrophied up to 20 days postoperation and then regressed. However, the type I fibres hypertrophied throughout the experimental period (100 days) studied. Unlike denervated eutherian hemidiaphragm, fibre-splitting was absent in the denervated marsupial muscle. An enhancement of the ATPase reaction in the denervated type I fibres may be due to dedifferentiation. Presumably innervation exerts a 'negative control' and prevents increase of type I fibre size in the normal hemidiaphragm.

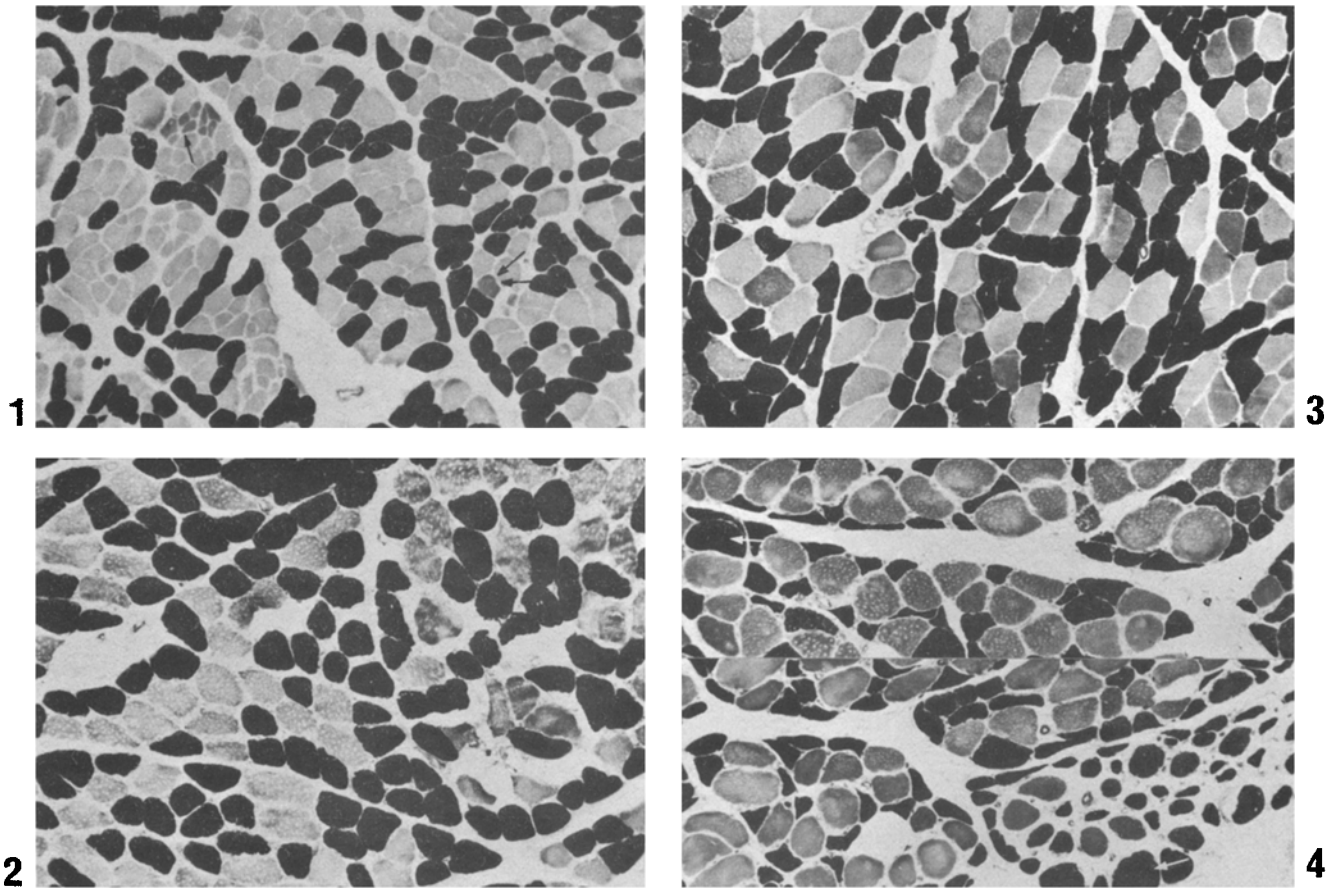
Vertebrate skeletal muscles atrophy when deprived of their innervation[2,3]; however, denervated rat hemidiaphragm shows a transient phase of hypertrophy for up to 10 days followed by atrophy[4-14]. Biochemical[6,7] and ultrastructural[8] studies confirmed an increase in contractile and sarcoplasmic proteins during hypertrophy. Further, the increase and decrease of proteins during hypertrophy and atrophy respectively paralleled the time course of an increase and decrease of RNA in the eutherian hemidiaphragm[7]. The purpose of this investigation was to evaluate the response of marsupial hemidiaphragm after denervation and to determine the histochemical behaviour of the 2 major muscle fibre-types sequentially.

Materials and methods. 12 quokkas *(Setonyx brachyurus)* of both sexes weighing between 1.97 and 3.45 kg were used in this study. The animals were anaesthetized with ether and intrathoracic denervation was performed and a 2 mm segment of the left phrenic nerve was removed[11]. On 4, 10, 20, 40, 60 and 100 days after phrenictomy, the normal innervated and the denervated muscles were removed carefully, cleared of all extraneous tissue and weighted. Small muscle blocks were excised from the normal and denervated hemidiaphragm muscles, and covered with talcum powder[15]. 10 µm frozen sections were cut on a cryostat and then incubated for the myofibrillar ATPase reaction[16].

Results. Compared with the innervated contralateral controls, the wet weight of denervated quokka hemidiaphragm increased: by 33.3% on day 4, 35.0% on day 10, 14% on day 20, 50% on day 40 and 12% on day 60. Thereafter its atrophy was 53% on the 100th day. Figure 1 shows that sizes of type I (pale staining) and type II (darkly stained) muscle fibres are similar. Unlike that seen in the rat hemidiaphragm[13,18], here the type I fibres were present in groups. Both type I and type II muscle fibres hypertrophied up to 20 days postdenervation (figures 2 and 3). Thereafter type II fibres regressed and atrophied; however, some hypertrophied and were still present on the 100th day (figure 4). The great majority of type I fibres hypertrophied throughout the experimental period (figures 2-4). Fibre splitting was absent in the denervated marsupial muscle during the postdenervation period studied (figure 4). In this denervated muscle, number of type IB (those with moderate ATPase reaction) appeared increased (figure 3). On the 100th day after surgery, all type I fibres displayed higher ATPase reaction (figure 4).

Discussion. Postdenervation hypertrophy of varying degree was evident in the denervated quokka hemidiaphragm up to 40 days. A previous report[11] also showed a 25-30% hypertrophy around day 40 in the denervated quokka hemidiaphragm; however, they did not study this denervated muscle prior to day 10. Here 33% and 35% hypertrophy of the hemidiaphragm were recorded on the 4th and 10th days, respectively, after phrenectomy.

As shown previously in the denervated rat hemidiaphragm[13], the type II fibres of quokka hemidiaphragm also hypertrophied up to 20 days postdenervation and then regressed. However, some hypertrophied type II fibres of this marsupial muscle were present in the atrophied tissue



Figures 1–4 are transverse sections showing the ATPase reaction; their magnification is identical (× 160).

Fig. 1. Normal quokka hemidiaphragm. Both type I (light) and type II (dark) muscle fibres are of similar size. A group of 10 small type IB (those with moderate ATPase reaction) fibres is indicated by an arrow. A single IB fibre of normal size is also present (2 arrows).

Fig. 2. 10 days after phrenic denervation. Note both type I and type II fibres are hypertrophied.

Fig. 3. 20 days postdenervation. Note both type I and type II fibres show increased size.

Fig. 4. 100 days postdenervation. The type I fibres are hypertrophied, but majority of type II fibres have atrophied. A few type II fibres (arrow) did not regress and atrophy. Fibre-splitting is not evident. One fascicle in right bottom corner is comprised of type II fibres predominantly. Note the increase of ATPase reaction in all type I fibres.

(i.e. on 100th day) indicating their resistance to regression, unlike that shown by the denervated type II fibres of the rat hemidiaphragm[12–14,17]. Interestingly, the type I fibres of denervated marsupial hemidiaphragm also differed in their behaviour in that they did not regress to near normal size, up to 100 days, as did the type I fibres of the rat[13,17].

The number of type IB[18] muscle fibres (those with moderate ATPase reaction) increased in the denervated quokka hemidiaphragm up to day 20. This indicates an increase in their ATPase activity and hence a conversion from type IA[18] to IB. Subsequently, all type I fibres displayed higher ATPase reaction. The denervated hemidiaphragm becomes subjected to periodic stretch/tension due to the rhythmic contraction of innervated contralateral hemidiaphragm[4–14]. Thus an increase of ATPase reaction in type I fibres seen here occurs due to passive stretch in the absence of neural mediation[9,19]. An increase in the ATPase activity of the denervated type I fibres of guinea-pig muscle was explained in terms of the dedifferentiation process[20]. Moreover, the nerve supply presumably exerts a 'negative control' and prevents size increase of the normal type I fibres.

Muscle fibre-splitting[21–23] (adaptive response to repair) occurs in the rat hemidiaphragm approximately 2 weeks after denervation[8,13,14,17]. However, these so-called regenerative muscle fibres are abortive in the rat muscle[8]. Fibre-splitting was absent in the marsupial hemidiaphragm even on 100th day after denervation. The reasons for this lack of regenerating capacity in this marsupial muscle are not known. It is possible, although unlikely, that 100 days on the time scale of postdenervation in hemidiaphragm of a larger animal quokka represents less than 2 weeks on the time scale of the denervated rat hemidiaphragm.

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Reduction of serum X-prolyl dipeptidyl-aminopeptidase activity in tumour-bearing mice and reversal of reduced enzyme activity by lentinan, an anti-tumour polysaccharide

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Summary. Serum X-prolyl dipeptidyl-aminopeptidase activity which had been shown to be depressed in cancer patients was clearly reduced in mice with Ehrlich carcinoma and Sarcoma 180, and slightly reduced in mice with methylcholanthrene-induced sarcomas. The reduced enzyme activity was completely reversed during tumour regression of sarcoma 180 by administration of lentinan, which causes regression of sarcoma 180.

X-Pro dipeptidyl-aminopeptidase (dipeptidyl-aminopeptidase IV), which hydrolyzes N-terminal X-Pro from peptides, is a dipeptidyl-aminopeptidase discovered in the rat liver and kidney by Hopsu-Havu and Glenner[1] by using a chromogenic substrate, Gly-Pro β-naphthylamide. This enzyme was found in the serum of various animals including the human[2-4]. The enzyme activity in the human serum was found to be decreased in patients with gastric cancer, pancreatic cancer, acute lymphocytic leukemia, lymphosarcoma and Hodgkin's disease, with Gly-Pro β-naphthylamide or X-Pro p-nitroanilides (X= Gly, Ala, Lys, Arg, Glu, and Asp) as substrate[3,5,6]. In order to clarify the significance of a decrease in the serum enzyme activity of cancer patients, we have examined the changes in serum X-Pro dipeptidyl-aminopeptidase activity in tumour-bearing mice with and without the administration of lentinan, a potent anti-tumour polysaccharide[7].

Materials and methods. C3H/CRJ and ICR/CRJ mice (females) were used for experiments. Lentinan, a β-(1 → 3) glucan isolated from a mushroom *Lentinus edodes* (Berk.) Sing., was prepared at Central Research Laboratories, Ajinomoto Co., INC. Autochthonous tumour was induced by s.c. administration of methylcholanthrene to C3H/CRJ mice at 11 weeks of age, and the serum enzyme activity was assayed at 28 days after the 1st observation of the tumours, the size of which being about 0.5 cm in diameter or 20 mm^2 in volume. Transplantable tumour-bearing mice were prepared with ICR/CRJ mice by transplantation of Ehrlich ascites carcinoma or Sarcoma 180 tumour (3×10^6 cells per mouse). Ehrlich ascites carcinoma was transplanted s.c. at 5 weeks of age, and mice were killed at 35 days after transplantation. Sarcoma 180 tumour cells were inoculated s.c. to 2 groups of mice at 5 weeks of age, then mice of 1 group were injected i.p. with 25 µg/mouse of lentinan daily for 10 days starting from day 1 after tumour inoculation, while the mice of the other group were not treated with lentinan. Mice were killed at 14, 21, 28 and 35 days after transplantation. We initially planned the experiments to consist of at least 4 mice for each group. However, we reduced the numbers of mice to 3 in Sarcoma 180 tumour-bearing mice in order to see the time course of the effects of lentinan. The diameter of the tumours was measured by vernier calipers and the surface area was calculated. X-Pro dipeptidyl-aminopeptidase activity was assayed by using a highly sensitive fluorescence assay with a newly synthesized substrate, 7-(Gly-Pro)-4-methylcoumarinamide[8,9]. The incubation mixture for X-Pro dipeptidyl-aminopeptidase contained (total volume 100 µl) 60 mM glycine-NaOH buffer (pH 8.7), 0.5 mM 7-(Gly-Pro)-4-methylcoumarinamide tosylate, and 10 µl of serum containing the enzyme. Serum was omitted during the incubation and added after stopping the incubation for the control tube. Incubation was carried out at 37 °C for 30 min, the reaction was stopped by adding 3.0 ml of 1 M sodium acetate buffer, pH 4.2, and the fluorescence intensity of 7-amino-4-methylcoumarin formed was read at 460 nm with excitation at 380 nm. In order to see whether or not the changes in serum X-Pro dipeptidyl-aminopeptidase activity are specific, the activity of serum leucine aminopeptidase, a similar enzyme with a broader substrate specificity, was also examined. Leucine aminopeptidase activity was assayed by using leucine β-naphthylamide as substrate, as described previously[10].

Results and discussion. The activities of X-Pro dipeptidyl-aminopeptidase and leucine aminopeptidase in the sera of tumour-bearing mice are shown in the table. The values of X-Pro dipeptidyl-aminopeptidase activity of normal mouse in the table are much higher than those described previously for the mouse (Nagatsu et al.[2]). The reason of this difference may be due to the differences in the mice strains and/or in the assay conditions. This assay was done at pH 8.7 (an optimum pH) with 7-(Gly-Pro)-4-methylcoumarinamide as substrate, whereas in the previous assay[2] Gly-Pro β-naphthylamide at pH 7.0 was used. Different normal values were also observed in the 2 mice strains under the same assay conditions in this study, indicating a strain difference of the enzyme activity.

A small reduction in serum X-Pro dipeptidyl-aminopeptidase