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Study on the Growth Limiting Mechanism in the Rat Ventral Prostate (39665)

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The male accessory sex glands are under the direct control of androgens, and the ventral prostate in rats is no exception. Thus this prostatic lobe is involuted after orchiectomy, an effect which depends on the activation of autophagic processes in the epithelial cells, resulting in autolysis and loss of functional performance (1, 2). Exogenous androgen to such rats will initiate DNA synthesis and cell proliferation. However, when the number of cells is restored to normal, DNA synthesis is curtailed and cell proliferation markedly reduced (3, 4). Hence, the proliferative growth of the prostate (but not its secretory ability) becomes refractory to even excessive androgen stimulation. This indicates the existence of a probable homeostatic constraint mechanism in the prostate.

Different possible inhibitory mechanisms have recently been discussed by Bruchovsky *et al.* (5), who considered the production of chalones (6) from the prostatic epithelial cells as an attractive possibility to explain the negative feedback on proliferative growth. If the prostatic epithelial cells produced a blood-borne tissue-specific growth inhibitor (a chalone) in a precise amount from a normal number of cells sufficient to shut off proliferation, then proliferation would occur only when the number of cells had fallen below the normal level and would be terminated in the regenerating prostate once the normal number of cells had been restored.

Support for the hypothetical existence of a prostatic chalone has been furnished by several clinical studies on the effects of a prostatic extract on patients with benign prostatic hyperplasia. Treatment with such an extract, possibly containing a growth-inhibitory prostatic chalone, was shown to give a significant symptomatic improvement in a considerable percentage of patients in the earlier stages of the disease but not in the later stages (7-10). Conclusive experimental results demonstrating the existence of a prostatic chalone are, however, still lacking, and the purpose of this study was to provide experimental evidence for the existence or nonexistence of a prostatic chalone regulating prostatic growth in rats.

Materials and methods. Adult male Wistar-Furth rats, 250-350 g, obtained from Microbiological Laboratories, Bethesda, Maryland, were used in the experiments. The animals, which are closely inbred, were kept under standard conditions and had free access to food and water.

In the first experiment 20 rats were divided at random into four groups. Half of the ventral prostate in the rats in two of the groups was extirpated under ether anesthesia. The extirpated part of the ventral prostate in each rat was weighed and its DNA content determined (11). The rats in the two other groups underwent sham operation. Two weeks and four weeks after the operation, the animals in one group with hemiprostatectomized rats and the animals in one group with sham operated rats were sacrificed. The ventral prostate and other accessory sexual glands were dissected and weighed. The DNA content in the ventral prostate was determined.

In the second experiment 30 rats were divided at random into six groups. The rats in group 1 underwent sham operation, those in groups 2, 3, and 5 were orchiectomized and sham operated, and those in groups 4 and 6 were orchiectomized and had half of their ventral prostate extirpated. The extirpated part of the ventral prostate of each of the rats in groups 4 and 6 was weighed and its DNA content determined. Two weeks after the operations the rats in group 2 were sacrificed and the ventral prostate and other accessory sexual glands were dissected and weighed. The DNA content in the ventral prostate was determined. The rats in groups 5 and 6 were injected intramuscularly with

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100 mg/kg of testosterone hexyloxyphenylpropionate (Andradurin, AB Leo, Helsingborg, Sweden), whereas the rats in groups 1, 3, and 4 received an intramuscular injection (2 ml/kg) of the solvent, isopropyl myristate (ICN Pharmaceuticals Inc., Plainview, New York). Two weeks after the injection, all rats were sacrificed and the ventral prostate and other accessory sexual glands were dissected and weighed. The DNA content in the ventral prostate was determined.

In the third experiment 30 rats were orchiectomized and divided at random into three groups. Two weeks after the operation, the rats in the first group were sacrificed. The atrophied ventral prostate was dissected, divided into its two lobes, and implanted into the spleen and between the muscles of the neck (one lobe at each location) of the rats in the second group. Thus, as a result of the implants each animal had more prostate cells than are normally found in orchiectomized animals. Small pieces of the liver were implanted into the spleen and between the muscles of the rats in the third group. The rats were injected intramuscularly with 100 mg/kg of testosterone hexyloxyphenylpropionate. Two weeks later the rats were sacrificed and the ventral prostate and other accessory sexual glands were dissected and weighed. Also the prostatic implants were dissected and weighed. The DNA content of the dissected prostatic tissue was determined. The prostatic implants in one rat of the ten in the second group were taken for histological examination.

Results. The results from the first experiment showed that even after as long a time as 28 days, the ventral prostate in hemiprostatectomized rats had not regenerated back to the weight of that in the control rats. The mean weight in the hemiprostatectomized animals was 170 mg with a standard error of ±13 mg, whereas in the control rats the weight was 289 ± 19 mg. Also the DNA content in the prostate was much lower in the hemiprostatectomized rats, 509 ± 28 μg, than in the control rats, 851 ± 63 μg. When the weight and DNA content of the extirpated part of the ventral prostate in the hemiprostatectomized animals was added to the weight and DNA content of the ventral prostate at sacrifice, the combined weight and the combined content did not differ significantly ($P > 0.05$) from the weight and DNA content of the ventral prostate in the control rats. This indicates that in fact no regeneration at all had occurred in the ventral prostate, even if the number of prostatic epithelial cells have been reduced to approximately 50% by the hemiprostatectomy. Similar results were obtained from the rats sacrificed 14 days after the operation.

In the second experiment orchiectomy was shown to cause a profound atrophy of the ventral prostate. The weight was reduced to 8 and 13% in the hemiprostatectomized and the sham-operated rats, respectively, and the DNA content to 12 and 20%, respectively, in comparison with the weight and DNA content of the ventral prostate in the control rats.

Androgen treatment of orchiectomized, sham-operated rats initiated 14 days after operation reversed the prostatic atrophy. The weight and DNA content of the ventral prostate, 321 ± 16 mg and 880 ± 27 μg, respectively, did not differ significantly from the corresponding weight and content in the sham-operated control animals, 279 ± 18 mg and 847 ± 21 μg. In orchiectomized, hemiprostatectomized rats, however, the androgen treatment brought the ventral prostate weight and DNA up to only half the normal level, 166 ± 9 mg and 431 ± 22 μg, a level that could be expected to be found in hemiprostatectomized but otherwise intact rats. When the weight and DNA content of the extirpated part of the ventral prostate was added to the weight and DNA content of the ventral prostate in the orchiectomized, hemiprostatectomized, androgen-treated rats, the combined weight and DNA content, 303 ± 15 mg and 800 ± 29 μg, did not differ significantly from the weight and DNA content in the orchiectomized, sham-operated, androgen-treated rats, 321 ± 16 mg and 880 ± 27 μg. The results tend to indicate that an androgen-induced restoration of the atrophied ventral prostate could be terminated long before the number of prostatic cells had been brought back to normal.

In the third experiment the number of prostatic cells was artificially increased by intrasplenic and intermuscular implantation

of atrophic ventral prostatic tissue. Androgen stimulation caused the implants to grow considerably. At sacrifice, the implants in both the spleen and the muscles were well developed (Fig. 1). The microscopically examined implants had a normal histological appearance with tall columnar epithelial cells with a clear Golgi area and with the acinar lumina filled with secretion. The implants were well vascularized, and any hormonal secretion from the prostatic epithelial cells could be expected to reach the general circulation.

The histologically observable stimulation of the prostatic implants was accompanied by a marked increase in weight and DNA content (Table I). The combined weight of the implants was approximately half the weight of the rat's own ventral prostate, and the DNA content was approximately two thirds. The weight and DNA content of the rat's own ventral prostate was found to be the same in the animals implanted with prostatic tissue as in the animals implanted with the control tissue (liver). This means that the rats with the prostatic implants had a much higher total content of prostatic tissue than the control rats in which the prostatic tissue had grown to a weight and DNA content that could be expected to be found in normal rats.

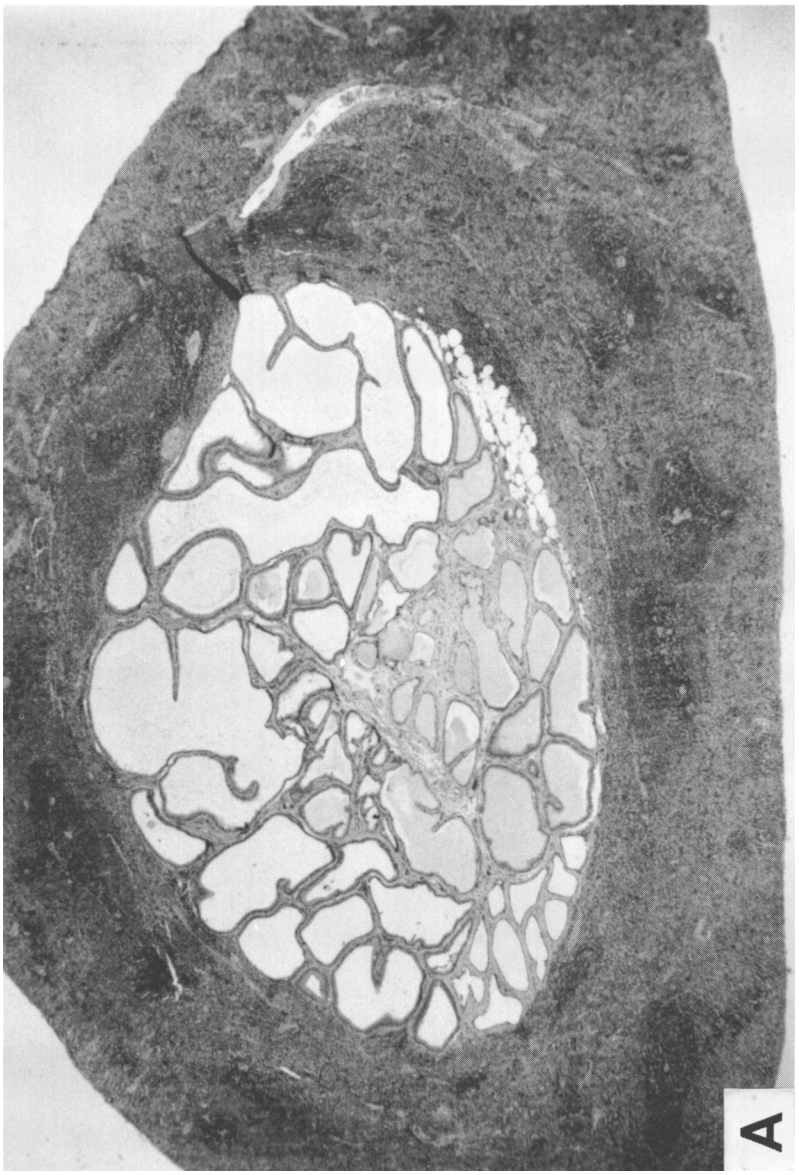
In all three experiments the weights of the dorsolateral prostate, coagulating glands, and the seminal vesicles were found to be unaffected by any surgically induced reduction or increase of the number of cells in the ventral prostate.

Discussion. The understanding of the regulation of growth and development of tissues and organs presents a great challenge for the biologist. Obviously, a regulatory mechanism is necessary for organ homeostasis, and it must play an important role in the response to injury and some unilateral organ extirpations such as nephrectomy. The homeostatic constraint mechanism is evidently defective in malignant tissue. The nature of the regulatory mechanism(s) has not been determined. One attractive theory is that tissue mitotic activity is controlled by a blood-borne tissue mitotic inhibitor, a chalone, in such a way that the greater the tissue mass within the body space, the lower will be the rate of cell production (6). This theory has recently been considered an attractive possibility to explain experimental results concerning the proliferation of prostatic epithelial cells (5). In the present study our aim was to obtain evidence for the existence of a prostatic chalone regulating the growth of that organ.

In the first of the three experiments the number of ventral prostatic cells was halved by hemiprostatectomy. If a chalone was secreted from the ventral prostate, the blood level of that mitotic inhibitor would be reduced. As a consequence, the remaining part of the ventral prostate could be expected to grow much in the same way as the liver would do after partial hepatectomy or the remaining kidney would do after unilateral nephrectomy (6, 12). The results clearly show, however, that no growth of the remaining part of the prostate had occurred 4 weeks after operation. This contrasts markedly to the regenerative ability of the rat liver where the normal size was regained within three weeks after removal of more than half of the tissue (13).

In the second experiment hemiprostatectomized, orchiectomized rats were given androgen to induce cell divisions and growth in the atrophied ventral prostate. If the prostate cells secreted a chalone regulating prostatic growth, cell division in the prostate of the hemiprostatectomized rats would not end until the normal cell number was restored. However, at the time of sacrifice, cell division had not progressed beyond the stage when the size and cell number was half that in the control rats and in normal rats.

In the third experiment the number of prostatic cells was increased above normal through implantation of ventral prostatic tissue from other rats. As we used closely inbred rats in all the experiments, there were no immunological difficulties, but the implants grew well after androgen treatment of the castrated recipient rats. If the prostatic cells secreted a chalone regulating prostatic growth, cell division in the ventral prostate of the implanted rats would be expected to end when the total number of prostatic cells in the body was back to normal. However, cell division in the ventral prostate of the implanted rats did not stop at that level but proceeded until the total number of prostatic cells (as reflected by the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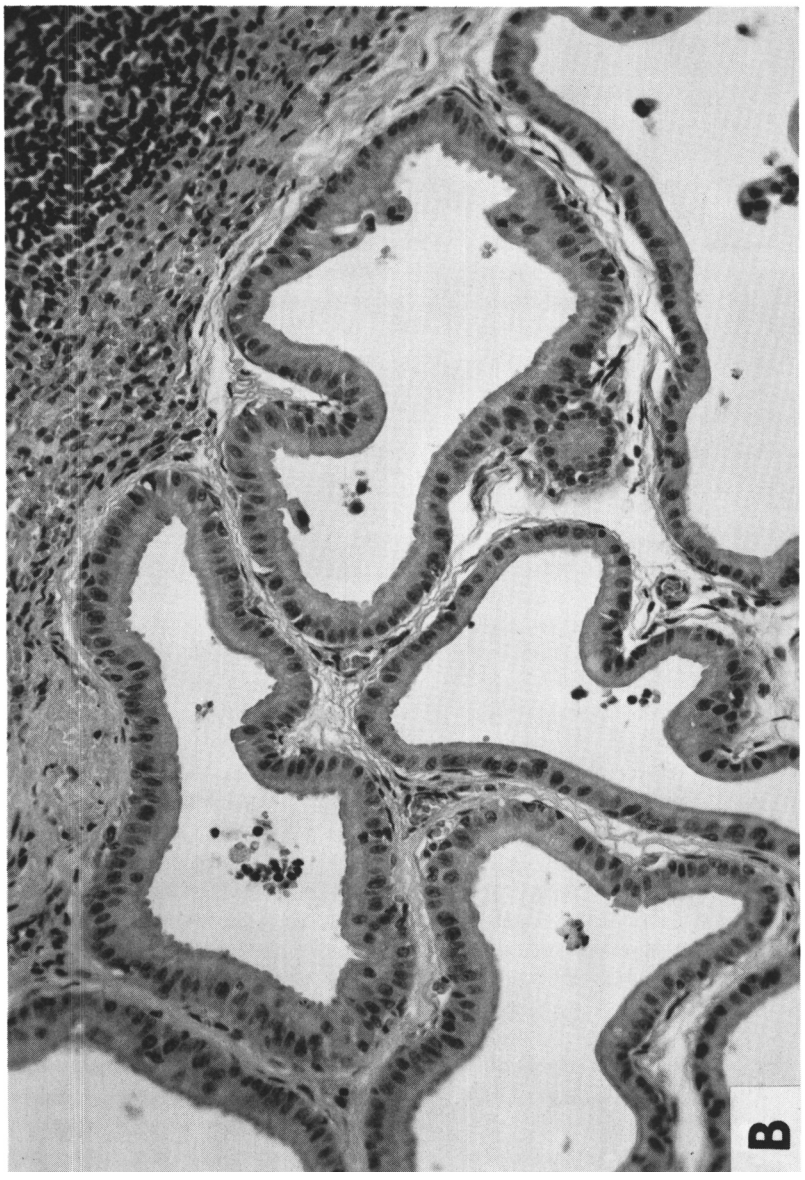

FIG. 1. Prostatic implant in the spleen 14 days after implantation into orchiectomized rats which then were given androgen. A. The implant which occupies the center part of the spleen has well developed secretion-filled acini with little surrounding fibromuscular stroma (×35). B. In most of the acini the epithelial cells are columnar and well developed with a clear Golgi area and a rounded nucleus with nucleoli (×280).

TABLE I. WEIGHT AND DNA CONTENT OF THE VENTRAL PROSTATE AND OF IMPLANTS OF VENTRAL PROSTATIC TISSUE INTO ORCHIECTOMIZED ANDROGEN-TREATED RATS.[a]

	Ventral prostate		Implants	
Group	Weight, mg	DNA content, μg	Weight, mg	DNA content, μg
A	338 ± 14	880 ± 22	165 ± 16	590 ± 62
B	316 ± 9	862 ± 17	–	–

[a] Orchiectomized rats received implants into the spleen and intermuscularly of the atrophied ventral prostate of other orchiectomized rats (Group A; $n = 9$) or of control tissue (liver) (Group B; $n = 10$). The animals were given a single injection of a long acting androgen, testosterone hexyloxyphenyl-propionate, and were sacrificed 14 days later. The mean values and their SE are given.

DNA content of the prostatic tissue) was 170% of the number in the control rats. At that level, the cell number in the ventral prostate of the implanted rats was the same as the cell number in the ventral prostate of the control rats. Thus, the results of these three experiments show that a prostatic chalone is not the only factor that can inhibit cell divisions in the prostate. In fact, a prostatic chalone seems to have at the most a very subordinate role in the mechanism regulating prostatic growth at least in the rat.

It is evident that further possible regulatory mechanisms must be considered and examined to find the factor that terminates the cell divisions in the prostate. This factor is evidently defective in the malignant prostate. It would appear that an increased knowledge of the regulatory mechanism may give new leads for alternative modes of treatment of prostatic cancer.

Summary. In search for the mechanism that limits the growth of the prostate, experiments were performed to find evidence for the existence of a prostatic chalone. Inbred rats were hemiprostatectomized to reduce the level of a hypothetical circulating chalone. No evidence of regeneration was found 4 weeks after operation. Other hemiprostatectomized, orchiectomized rats were given androgen to induce prostatic growth. Cell division in the ventral prostate ended when the size of the prostate was only half of that in the control rats. Orchiectomized rats with intrasplenic and intermuscular implants of ventral prostatic tissue from other orchiectomized rats were given androgen. The cell divisions in the prostatic tissue did not end until the total cell number was 170% of that in the control rats. The results indicate that other factors than a prostatic chalone have the dominating role in the mechanism that limits prostatic growth.

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