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NEONATAL EXPOSURE TO OESTROGENS ALTERS THE PROTEIN PROFILES AND GENE EXPRESSION IN THE GENITAL TRACT OF ADULT MALE MICE

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Summary—After neonatal administration of supraphysiological doses of oestradiol, the concentration of tissue proteins, in adult mice, was significantly reduced by 39, 45 and 56% in epididymis, vas deferens and seminal vesicle respectively. The protein profiles showed persistent alterations. In epididymis, 4 protein bands were differentially increased (14.4, 43 and 67 kDa) or reduced (24 kDa) in oestrogenized males. In vas deferens, 4 proteins were increased (14.4, 49,67 and 76 kDa) and one (34 kDa) virtually absent. In seminal vesicle, about 20 proteins of varying molecular weights (12–140 kDa) were differentially increased or decreased. Testosterone substitution, at adulthood, was unable to reverse these effects. Treatments with oestradiol during adult life induced persistent alterations in the protein profiles of the 3 organs but, in contrast to neonatal treatment, these alterations could be reversed by androgen therapy. A cDNA library has been constructed with RNA prepared from adult seminal vesicle and screened by differential hybridization. Neonatal oestrogenization strongly reduced the abundance of some mRNA species. Eleven recombinants containing putative oestrogen-sensitive sequences were isolated. Two of them, having an insert of about 500 base pairs, were used for dot-blot hybridization. Results showed that the two clones contained sequences which were differently regulated by androgens.

INTRODUCTION

It is well established that androgens regulate the growth, differentiation and function of male accessory sex organs [1]. In addition to testosterone, oestrogens have been measured in the plasma of males in a variety of species including rat and man [2] and it has been shown that in rat, circulating oestradiol levels were higher in immature than in adult males [3]. Specific oestradiol receptors have been described in male sex accessory organs of rat [4], guinea-pig [5] and rabbit [6]. Recent studies in rabbit [7] and rat [8] have shown that sex accessory glands of immature males are sensitive to oestradiol, suggesting that oestrogens may have a role in the functional development of these glands. However, excessive amounts of these compounds have adverse effects. Previous investigations have shown that if intact newborn male rats are exposed to exogenous oestrogens they show, at puberty, permanent structural and/or functional deficiencies characterized by infertility, atrophy of accessory sex organs, abnormal differentiation [9–13] and decreased amounts of specific secretory proteins from seminal vesicle and prostate [14]. Prenatal or neonatal exposure to these hormones as well as elevated circulating maternal oestrogens have also been associated with preneoplastic and neoplastic lesions of the male reproductive tract in mice [15–18] and humans [19,20]. The mechanisms underlying the toxicity of these compounds are not known and it has been suggested that they permanently alter the differentiation of reproductive tracts [21].

The present study represents an attempt: (a) to investigate the possibility of persistent altered differentiation of accessory sex organs as detected by changes in their protein profiles; (b) to determine the ability of androgens to reverse the effects of neonatal oestrogen administration; (c) to compare the changes induced in accessory sex organs by neonatal oestrogenization with those elicited in adult male submitted to the same treatment; and (d) to use recombinant DNA techniques to determine the effects of neonatal oestrogenization on the abundance of some mRNA species.

EXPERIMENTAL

Animals

Mice of the Swiss strain (CD-1 Charles River France) were raised under standardized conditions. One or 10 μg of oestradiol-17β (Theramex Laboratories, Monaco) dissolved in sesame oil were injected every day from 1 to 10 days of age. Treatment with 10 μg was equivalent to about 60 μg per 10 g body

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weight in newborns and to 15 μg per 10 g body weight in 10-day-old males. Animals were injected subcutaneously with a needle inserted at the mid-dorsal region of the neck, care being taken to prevent leakage of the solution. Animals were killed at 20, 30 and 110 days of age. A second experiment was designed to test the effect of oestradiol treatment from 1 to 10 days on the subsequent androgen responsiveness of the accessory sex organs in adulthood. Males treated as described above were castrated at 75 days of age; three weeks later some animals were killed and the others were injected with testosterone heptylate (75 μg twice daily) for 2 weeks and killed 12 h after the last injection. Adult males (3 months) were injected for 10 days with oestradiol-17β (120 μg daily, equivalent to 35 μg per 10 g body weight) and killed 100 days later. The responsiveness of the accessory sex organs of these males to testosterone was measured as described above. At the time of autopsy the epididymis, vas deferens and seminal vesicle were dissected out carefully and their weights were recorded accurately.

Protein assay

The organs were homogenized for 30 s at 4°C in 8 vol buffer A (0.25 M sucrose; 3 mM $MgCl_2$; 25 mM Tris; 0.5 mM phenyl-methyl-sulfonyl-fluoride, pH 7.5) in a glass–glass hand held homogenizer. Protein determination was performed in the 12,000 *g* supernatant [22] with bovine serum albumin (fraction V) as the reference standard.

Protein electrophoresis

One dimensional electrophoresis was performed under denaturing conditions using the procedure of Laemmli[23]. SDS protein samples (70–100 μg) were applied to 15% resolving gels (epididymis, vas deferens) or to 10–20% linear gradient resolving gels (seminal vesicle) with a 4.5% stacking gel and run at 25 mA at room temperature until the tracking dye (bromophenol blue) reached the bottom of the gel (about 30 min). Gels were then stained with Coomassie Brilliant blue.

Preparation of mouse seminal vesicle cDNA library

Total cellular RNA was isolated from adult mouse seminal vesicle by the guanidium isothiocyanate method [24]. Poly(A)$^+$ RNA enriched by oligo-dT cellulose chromatography [25] was used as a template for reverse transcriptase and the cDNAs were converted into double-stranded molecules by using the cDNA synthesis system (Amersham) following the protocol given by the manufacturer. Double-stranded cDNA was methylated, Eco RI linkers were added and the cDNA was cloned into lambda gt11 [26]. A total of 28,000 recombinant clones were plated at 300 plaque-forming units per 9 cm Petri dish with *Escherichia coli* 1090 and replicated on nitrocellulose filters. Single-strand cDNA probes were synthesized from the poly (A)$^+$ RNA extracted from seminal vesicles of control and neonatally oestrogenized males, using reverse transcriptase. DNA was labelled with [^{32}P]dCTP (20 μCi) and filters were hybridized with approximately 300,000 dpm of each probe [27]. The differences in hybridization observed between the "control" and the "neonatally oestrogenized" cDNA probes represent the cloned cDNA sequences which are complementary to the mRNA species whose abundance is affected by neonatal oestrogenization. Colonies hybridizing differently to the two probes were picked and rescreened 2 times.

RNA dot-blots

RNA dot-blot experiments were performed as described [28]. Nitrocellulose filters ruled into 1 cm squares were saturated with 20 × SSC (3 M NaCl, 0.3 M trisodium citrate, pH 7.0) air dried and spotted with different concentrations of total RNA which had been denatured by heating to 65°C for 15 min and rapidly cooled on ice. The filters were baked at 80°C for 2 h.

Radioimmunoassays

Testosterone was measured according to the method previously described [29].

Statistics

Values are means ± SEM. The significance of differences between means was calculated using Student's *t*-test. Differences were considered to be significant when $P < 0.05$.

RESULTS

Accessory sex organs size

Whereas the injection of 1 μg oestradiol from 1 to 10 days of age did not affect the weight of accessory sex organs, the administration of 10 μg markedly suppressed their growth (Table 1). The weights of

Table 1. Effects of neonatal oestrogenization on growth of accessory sex organs of 110 day old male mice

Animal group	Epididymis			Vas deferens			Seminal vesicle		
	Weight (mg)	Protein (μg)	Protein (μg/mg)	Weight (mg)	Protein (μg)	Protein (μg/mg)	Weight (mg)	Protein (μg)	Protein (μg/mg)
Intact males	102 ± 3	3900 ± 310	38 ± 2	34 ± 1	1800 ± 130	53 ± 2	420 ± 24	27,000 ± 2650	64 ± 3
Oestradiol (1 μg)	99 ± 4	5500 ± 190*	55 ± 1*	40 ± 3	2100 ± 120	54 ± 1	345 ± 37	21,000 ± 3000	60 ± 4
Oestradiol (10 μg)	72 ± 10*	1500 ± 320*	23 ± 2*	26 ± 3*	700 ± 90*	29 ± 2*	37 ± 5*	1200 ± 300*	28 ± 5*

Values are means ± SEM. $N = 10$.
*$P < 0.001$ compared with intact males.
Oestradiol-17β (1 μg or 10 μg) was administered every day from 1 to 10 days of age.

Table 2. Effects of neonatal oestrogenization on the subsequent responsiveness of accessory sex organs to testosterone administration in adult castrated males as compared to controls not given oestradiol treatment

Animal group	Epididymis			Vas deferens			Seminal vesicle		
	Weight (mg)	Protein (μg)	(μg/mg)	Weight (mg)	Protein (μg)	(μg/mg)	Weight (mg)	Protein (μg)	(μg/mg)
I	51 ± 1	1900 ± 120	38 ± 2	25 ± 1	1200 ± 60	47 ± 1	280 ± 11	13,500 ± 950	46 ± 2
II	62 ± 7	2500 ± 400	41 ± 2	29 ± 2	1300 ± 100	48 ± 2	220 ± 15*	11,000 ± 1100	50 ± 4
III	70 ± 6*	2900 ± 300*	43 ± 2	29 ± 2	1250 ± 150	44 ± 4	95 ± 15*	3400 ± 700*	34 ± 3*

I: males castrated at adulthood for 3 weeks and then treated for 2 weeks with testosterone heptylate.
II: males treated neonatally with 1 μg oestradiol, castrated at adulthood for 3 weeks and then treated for 2 weeks with testosterone heptylate.
III: males treated neonatally with 10 μg oestradiol, castrated at adulthood for 3 weeks and then treated for 2 weeks with testosterone heptylate.
Values are means ± SEM ($N = 10$).
*$P < 0.001$ compared to group I.

epididymis, vas deferens and seminal vesicle were 71, 79 and 9% of respective controls. The concentrations of proteins were significantly reduced by 39, 45 and 56% in the same organs, respectively.

Plasma testosterone levels

Circulating testosterone levels in adult males neonatally treated with 10 μg oestradiol (4.3 ± 1.7 nmol/l) were lower than in controls (20 ± 8 nmol/l) whereas those of males treated with 1 μg were unaffected (12 ± 5 nmol/l).

Responsiveness of the accessory sex organs to testosterone in adult mice

The responsiveness of epididymis and vas deferens of males oestrogenized with the lowest dose of oestradiol was unaffected whereas that of seminal vesicle was slightly reduced as judged by its weight (Table 2). In male mice treated neonatally with 10 μg oestradiol the injection of testosterone at adulthood increased the weight of epididymis so that this organ was larger than that of untreated males castrated and injected with testosterone. The responsiveness of vas deferens was similar to that of controls as judged by weight, protein content and concentration. In contrast, the seminal vesicles of oestrogenized males appeared to be poorly responsive to testosterone since the weight protein content and protein concentration of these glands were only 34, 25 and 74% of respective castrated-reinjected controls.

Protein pattern of sex accessory organs

The protein patterns of the 3 organs studied were differently affected by neonatal oestrogenization (10 μg). There was little quantitative or qualitative differences between epididymis from control or oestrogenized males (Fig. 1). The major effect was observed on 4 proteins which were increased (14.4, 43, 67 kDa) or reduced (24 kDa) in oestrogenized males. In vas deferens, protein bands with molecular weights of 14.4, 49, 67 and 76 kDa were increased whereas the 34 kDa protein band, which was a major band in controls, was virtually absent (Fig. 1). In seminal vesicle, within the 50–60 bands revealed in Coomassie blue-stained gels, about 20 of them were differently affected (Fig. 1). Nine bands with a molecular weight of 12, 13, 14, 15, 15.5, 34, 36, 120 and 140 kDa respectively were strongly reduced or virtually absent in oestrogenized males. Nine bands with a molecular weight of 12.5, 14.3, 18, 22, 26, 43, 53, 67 and 78 kDa respectively, which were slightly apparent in controls, were strongly increased in oestrogenized males. In the 90–105 kDa region, several weak bands were also increased in treated males. In all these studies we have included only those proteins which, by visual examination, varied reproducibly in 3 separate experiments.

To determine the onset of these alterations the protein patterns of the accessory sex organs of neonatally oestrogenized males were examined at 20 and 30 days of age. At 20 days, the protein profiles of the 3 organs were similar in control and treated males except for the 67 kDa band which was increased in the 3 organs and for the 34 kDa band which was missing in the vas deferens of oestrogenized males (not shown). When the neonatally oestrogenized males were examined at 30 days, most of the alterations described in adult males were already present (not shown).

In order to determine if the alterations described above were consecutive to cumulative effects of long-term impaired testosterone secretion, adult untreated males and neonatally oestrogenized males were castrated (3 weeks) and injected (2 weeks) with testosterone heptylate. Detailed comparison of Coomassie blue-stained gels revealed that the alterations observed in the electrophoretic pattern of proteins from the 3 organs of oestrogenized males (Fig. 1) persisted after testosterone substitution at adulthood, indicating that testosterone was unable to reverse the effects of neonatal oestrogenization (Fig. 2).

Treatment of adult males for 10 days with oestradiol caused, in the protein profiles of the 3 organs, persistent alterations similar to those described in adult neonatally oestrogenized males. When these adult oestrogenized males were castrated and 3 weeks later injected for 2 weeks with testosterone heptylate, the protein profiles of the 3 organs were, except for small differences, similar to those of controls. The protein profiles of seminal vesicle are shown as an example in Fig. 3.

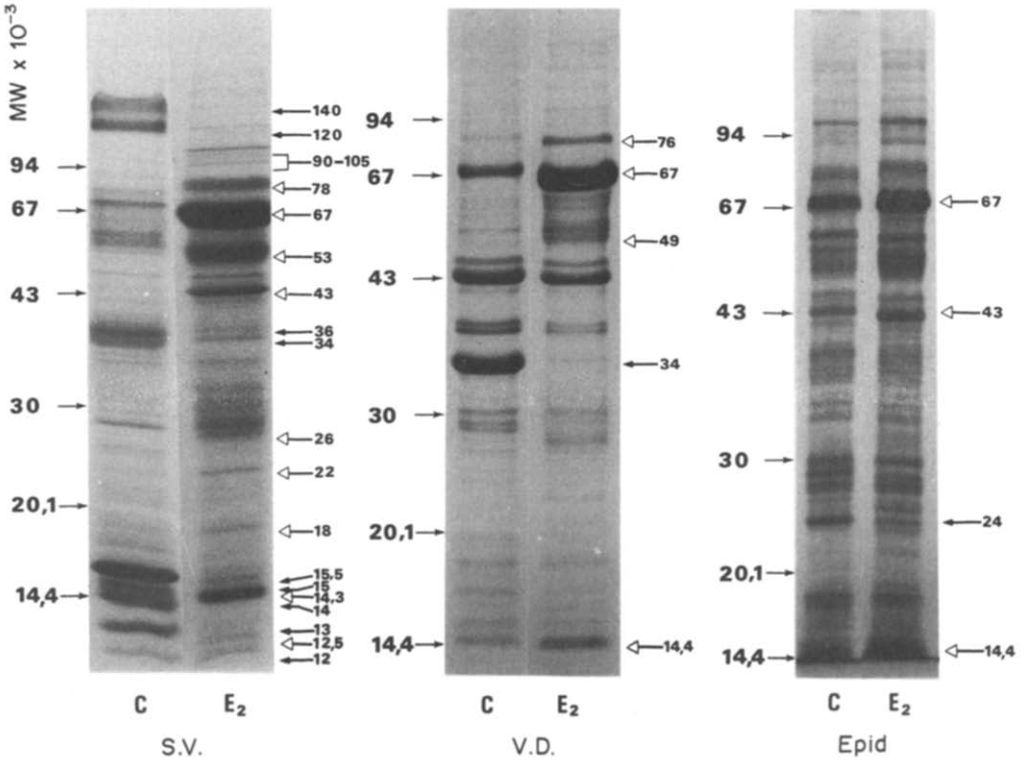

Fig. 1. Analysis by SDS–PAGE of proteins from epididymis (Epid), vas deferens (V.D.) and seminal vesicle (S.V.) of adult mice. Each lane was loaded with 70 μg proteins (epididymis, vas deferens) or 100 μg proteins (seminal vesicle). Proteins were stained with Coomassie Blue. E_2: neonatally oestrogenized group. C: control group. White arrows indicate the position of the protein bands increased by neonatal oestrogenization. Black arrows indicate the position of the protein bands decreased by neonatal oestrogenization.

Isolation of RNA species affected by neonatal oestrogenization

As shown above seminal vesicle is more affected than epididymis and vas deferens by neonatal oestrogenization. For this reason a cDNA library was prepared from mRNA isolated from adult mouse seminal vesicle. In order to isolate cDNA clones corresponding to oestrogen-responsive proteins, 25,000 individual colonies were screened by differential hybridization. Labelled cDNA was synthesized from poly $(A)^+$ RNA prepared either from control or from adult neonatally oestrogenized males. Colonies that gave hybridization signals of different intensity with the two probes were put through a second round of differential hybridization. The results of screening the cDNA library showed that a large number of false positives were taken through the second round. An example of differential screening is shown in Fig. 4. The recombinants hybridized with a range of intensities, probably reflecting the abundance of the different RNAs. A few number of recombinants hybridized significantly more strongly with cDNA derived from controls than with cDNA from neonatally oestrogenized males. Among the 300 recombinants which were submitted to a third round of differential screening, 11 of them, containing putative oestrogen sensitive sequences were isolated. Two of them (clones E1, E2) having an insert of about 500 base pairs were used for a more extensive examination. The labelled inserts were used in dot-blot hybridization against increasing concentrations of seminal vesicle RNA isolated from intact adult male controls, neonatally oestrogenized males, adults castrated 3 days before and adults castrated for 3 days then treated with testosterone heptylate (75 μg twice daily for 4 days). Figure 5 shows that the hybridization signal observed in controls was strongly reduced in neonatally oestrogenized males. Such positive clones would contain cDNA sequences which are complementary to mRNA species abundant in the normal seminal vesicle but scarce in the seminal vesicle of neonatally oestrogenized males. When the clone E1 was used against mRNA isolated from adult castrated males, the signal obtained was strongly reduced and it was restored by androgen substitution. When the clone E2 was used in the same conditions the signal was weakly reduced by castration and partly restored by androgen therapy.

DISCUSSION

The results reported here agree with earlier studies showing that supraphysiological doses of oestrogens administered to male rats within the first neonatal

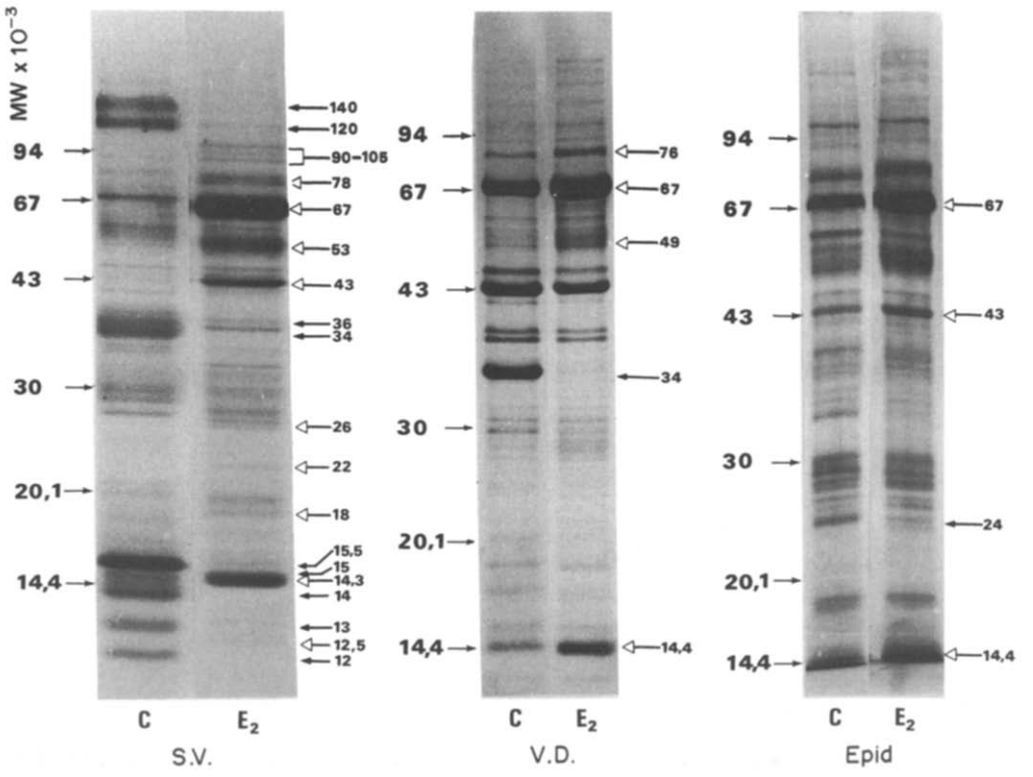

Fig. 2. Analysis by SDS–PAGE of proteins from epididymis (Epid), vas deferens (V.D.) and seminal vesicle (S.V.) of adult mice. Each lane was loaded with 70 μg (epididymis, vas deferens) or 100 μg proteins (seminal vesicle). Proteins were stained with Coomassie blue. E_2: neonatally oestrogenized mice castrated at adulthood for 3 weeks and then treated for 2 weeks with testosterone heptylate. C: untreated mice castrated at adulthood for 3 weeks and then treated for 2 weeks with testosterone heptylate. White arrows indicate the position of the protein bands increased by neonatal oestrogenization. Black arrows indicate the position of the protein bands decreased by neonatal oestrogenization.

days resulted in a definitive reduction in the weight of accessory sex organs in adulthood [9, 12, 30]. In addition, the present results indicated that the reduction of the weight of accessory sex organs was paralleled by a decrease in protein concentration. Within the 3 organs studied, the seminal vesicle was the least responsive to endogenous or exogenous androgens. The inhibition of accessory sex organs growth might be due to direct effects of the injected oestrogen and/or to cumulative effects of long-term impaired testosterone secretion. During the neonatal and weaning period the presence or absence of androgens affects not only the immediate developmental events, but also the response of accessory sex organs such as prostate and seminal vesicles to androgens in adulthood [30–32]. Then, the altered growth response that we observed in adult neonatally oestrogenized males could be a result of earlier perturbations in androgen secretion. Neonatal oestrogenization might also result in impairment of morphological differentiation and/or alteration in biochemical processes such as deficiencies in 5α-reductase, androgen receptors or post-receptors defects. Stromal–epithelial interactions are known to be of utmost importance during embryonic and postnatal development [1]. In the prostate and seminal vesicle the mesenchyme induces epithelial differentiation and androgens elicit these effects by acting primarily on the stromal cells which elicit epithelial proliferation through putative trophic factors [33]. Most investigators have found that the fibro-muscular stroma responds to oestrogens and the epithelium to androgens [34, 35] according to the selective localization of androgen and oestrogen receptors in these compartments [4]. Since neonatal DES exposure caused significant increase in the cytosolic oestrogen receptor levels in mouse seminal vesicle [36], neonatal oestrogenization could induce hypertrophy of the musculo-fibrous components as it has been shown in accessory sex organs of rabbits [7] and rats [11, 12, 34]. Therefore in organs in which the ratio of epithelium to stroma is altered, androgen receptors and protein synthesis could be influenced by different cell types present during testosterone induced growth. It is also possible, although not documented, that neonatal oestrogenization induces qualitative or quantitative changes in androgen receptors. Persistent receptor changes induced by glucocorticoids [37], cyproterone acetate [38, 39] and oestrogens [36, 40] administered neonatally have been reported in mice and rats. In mice, neonatal DES exposure caused a significant decrease in cytosolic androgen receptor levels in the anterior prostate [36].

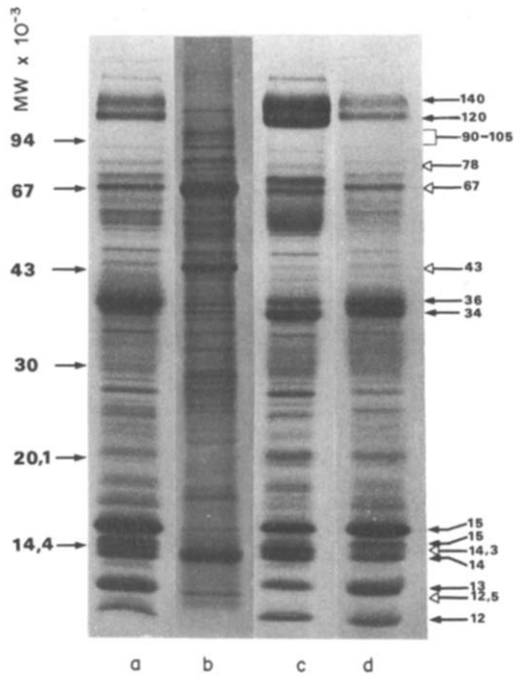

Fig. 3. Analysis by SDS–PAGE of proteins from seminal vesicles of adult mice (7 months). Each lane was loaded with 100 μg proteins. Proteins were stained with Coomassie Blue. (a) Control males, (b) adult males treated 10 days with oestradiol-17β (120 μg daily) and killed 100 days later, (c) untreated mice castrated at adulthood for 3 weeks and then treated for 2 weeks with testosterone heptylate, (d) adult males oestrogenized as described above, castrated for 3 weeks and then treated for 2 weeks with testosterone heptylate. White arrows indicate the position of the protein bands increased by adult oestrogenization. Black arrows indicate the position of the protein bands decreased by adult oestrogenization.

Neonatal administration of oestrogens strongly reduced circulating testosterone levels in immature [41] and adult animals (present results). It has been shown that in prostate [42] and seminal vesicle [43] testosterone exerted a more conspicuous effect on rRNA synthesis which may be a key event in protein synthesis and cellular growth. These processes could be, at least partly, altered by the neonatal suppression of androgen secretion in oestrogenized males, leading to a reduced efficacy of testosterone in adulthood.

This report describes persistent altered differentiation following exposure to oestradiol as detected by changes in protein profiles of accessory sex organs. Some of these proteins (14.4, 43 and 67 kDa) had the same mobility as α-lactalbumin, ovalbumin and albumin respectively and were probably not specific proteins since they are present in several organs. Most interesting were the proteins which could be identified with tissue specific androgen marker proteins previously described in accessory sex organs of mouse [44, 45]. In the vas deferens, the 34 kDa protein band, which was missing in oestrogenized males, corresponds probably to the major androgen-dependent protein previously described [44]. Based on colour staining and apparent molecular weight, some protein bands (12, 13, 14, 15.5, 120 and 140 kDa respectively) which were reduced in seminal vesicle from oestrogenized males could be identified with androgen-induced proteins described in adult mice [45]. Similarly, some protein bands (12.5, 14.3, 53 and 90–105 kDa respectively) which were increased in seminal vesicle from oestrogenized males, correspond probably to androgen-repressed proteins of the same molecular weights [45]. Since in the rat seminal

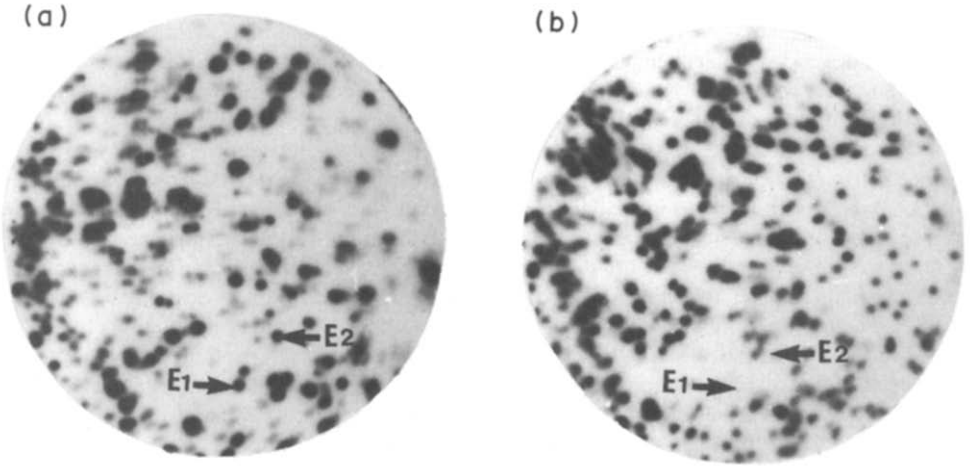

Fig. 4. Detection of recombinants containing oestrogen-responsive sequences by differential screening of seminal vesicle cDNA library (primary screening). Bacterial colonies were grown on nitrocellulose filters, lysed and immobilized. The filters were hybridized to reverse transcribed poly $(A)^+$ RNA from control seminal vesicle (a) and from seminal vesicle of oestrogenized males (b). Arrows indicate recombinants that hybridized more strongly with cDNA derived from controls than with cDNA from neonatally oestrogenized males.

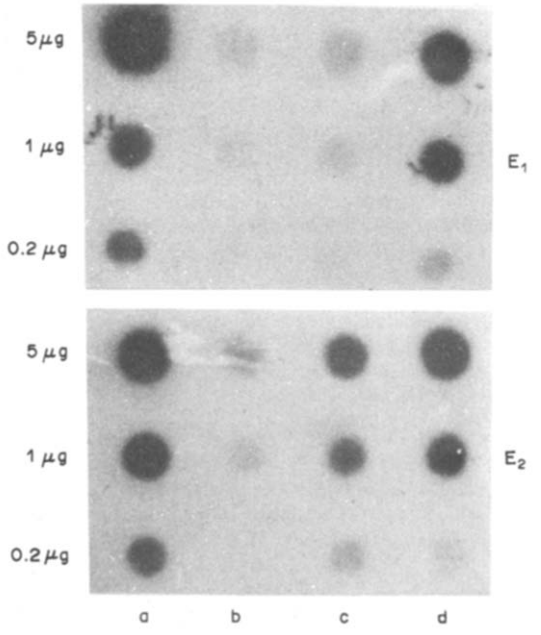

Fig. 5. Dot-blot analysis of seminal vesicle RNA extracted from normal and neonatally oestrogenized males in response to castration and testosterone substitution. Total RNA from controls (a), neonatally oestrogenized males (b), adult castrated males (c), and castrated males injected with testosterone (d), were spotted onto nitrocellulose and hybridized with ^{32}P-labelled E_1 and E_2 probes. The amount of RNA spotted was 5 μg and subsequent serial dilutions were 1:5.

vesicle [14] and mouse vas deferens [46] specific androgen marker proteins are synthesized by epithelial cells, the modifications we observed in protein/gene expression were probably due to changes in glandular epithelium rather than in stroma. The alterations of protein profiles of the 3 accessory sex organs studied were yet present at 30 days of age. Since in controls the androgen-regulated proteins appeared (induced proteins) or disappeared (repressed proteins) between 20 and 40 days [44, 45], we may conclude that neonatal oestrogenization inhibits these processes.

The alteration of the synthesis of these proteins might not be attributable to the cumulative effect of long-term impaired testosterone secretion since the effects could not be reversed by adult testosterone therapy. In contrast, oestrogenization of adult males induced similar alterations in the protein profiles of the 3 accessory sex organs which could be reversed by androgen substitution therapy. This indicates that the neonatal period is particularly critical not merely for growth and morphological development of accessory sex organs [9, 12, 32] but also for the functional activity of vas deferens and seminal vesicle in adulthood. Previous studies, in rats, have shown that neonatal oestrogenization strongly reduced protein synthesis and secretory capabilities of prostate and seminal vesicle [14]. However, whether androgen substitution in adulthood restores normal protein synthesis is unknown.

We have shown, by differential hybridization, that neonatal oestrogenization reduced, in adulthood, the abundance of some mRNA species. The identity of any proteins that might be encoded in the recombinants selected from the seminal vesicle cDNA library is as yet unknown. When one of these clones (E1) was used in dot-blot hybridization against mRNA isolated from neonatally oestrogenized males and from adult castrated ones, the signal obtained was strongly reduced in both cases suggesting that some oestrogen-responsive sequences correspond to androgen-induced RNAs. However clone E2 contains sequences which were less sensitive to androgens. Then, the alterations observed in protein profiles might be probably attributable, at least for some of them, to changes in their mRNA levels. There are very few studies concerning persistent changes, at mRNA level, induced by prenatal or neonatal oestrogenization. It has been shown that the mRNA for the major inducible-uterine secretory protein (lactotransferrin) was expressed in the seminal vesicle of male mice prenatally exposed to DES but not in controls [47]. However, the ability of these seminal vesicles to synthesize messages for an androgen-dependent protein (SVS IV) is maintained [47]. It is well known that transcribed genes reside in domains of an altered chromatin structure in which the DNA is in a so-called "open configuration state" (reviewed in Ref. [48]). Such a chromatin conformation can be recognized by the accessibility of genomic DNA to nucleases and a correlation between DNAse I sensitivity and gene expression has been established (reviewed in Ref. [49]). The gradual conversion from a "closed conformation" to an "open conformation" of some chromatin domains, which occurs during fetal and neonatal development under hormonal influences [49] could be, at least partly, prevented by neonatal oestrogenization. Then, in adulthood, the accessibility of specific high affinity sites of chromatin could be limited leading to an altered expression of some mRNA species.

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REFERENCES

1. Cunha G. R., Donjacour A. A., Cooke P. S., Mee S., Bigsby R. M., Higgins S. J. and Sugimura Y.: The endocrinology and developmental biology of the prostate. *Endocr. Rev.* **8** (1987) 338–362.
2. Van Der Molen H. J., Brinkmann A. O., De Jong F. H. and Rommerts F. F. G.: Testicular oestrogens. *J. Endocr.* **89** (1981) 33–46.
3. Döhler K. D. and Wuttke W.: Changes with age in levels of serum gonadotropins, prolactin and gonadal steroids in prepubertal male and female rats. *Endocrinology* **97** (1975) 898–907.
4. Jung-Testas I., Groyer M. T., Bruner-Lorand J., Hechter O., Baulieu E. E. and Robel P.: Androgen and estrogen receptors in rat ventral prostate epithelium and stroma. *Endocrinology* **109** (1981) 1287–1289.

5. Danzo B. J., Raymond P. A. and Davies J.: Hormonally responsive areas of the reproductive system of the male guinea pig. III. Presence of cytoplasmic estrogen receptors. *Biol. Reprod.* **25** (1981) 1159–1168.
6. Danzo B. J., Eller B. C., Judy L. A., Trautman J. R. and Orgebin-Crist M. C.: Estradiol binding in cytosol from epididymides of immature rabbits. *Molec. Cell. Endocr.* **2** (1975) 91–105.
7. Orgebin-Crist M. C., Eller B. C. and Danzo B. J. The effects of estradiol, tamoxifen, and testosterone on the weights and histology of the epididymis and accessory sex organs of sexually immature rabbits. *Endocrinology* **113** (1983) 1703–1715.
8. Setty B. S., Jehan Q., Srivastava A. and Kamal N.: Biological response of the rat epididymis to estrogen. *Exp. Clin. Endocr.* **87** (1986) 229–238.
9. Kincl F. A., Folch Pi A. and Herrera Lasso L.: Effect of estradiol benzoate treatment in the newborn male rat. *Endocrinology* **72** (1963) 966–968.
10. Jean-Faucher Ch, Berger M., De Turckheim M., Veyssière G. and Jean Cl.: Effets de l'injection néonatale d'oestradiol, de testosterone et d'acétate de cyprotérone sur la testosterone testiculaire et plasmatique et sur l'appareil génital de la souris mâle adulte. *Arch. Anat. Microsc. Morph. Exp.* **65** (1976) 37–46.
11. Rajfer J. and Coffey D. S.: Sex steroid imprinting of the immature prostate. Long-term effects. *Invest. Urol.* **16** (1978) 186–190.
12. Chung L. W. K. and MacFadden D. K.: Sex steroids imprinting and prostatic growth. *Invest. Urol.* **17** (1980) 337–342.
13. Arai Y., Mori T., Suzuki Y. and Bern A. A.: Long-term effects of perinatal exposure to sex steroids and diethylstilbestrol on the reproductive system of male mammals. *Int. Rev. Cytol.* **84** (1983) 235–268.
14. Higgins S. J., Brooks D. E., Fuller F. M., Jackson P. J. and Smith S. E.: Functional development of sex accessory organs of the male rat. Use of oestradiol benzoate to identify the neonatal period as critical for development of normal protein-synthetic and secretory capabilities. *Biochem. J.* **194** (1981) 895–905.
15. Dunn T. B. and Green A. W.: Cysts of the epididymis, cancer of the cervix, granular cell myoblastomas and other lesions after estrogen injection in newborn mice. *J. Natn. Cancer Inst.* **31** (1963) 425–455.
16. McLachlan J. A., Newbold R. R. and Bullock B. C.: Reproductive tract lesions in male mice exposed prenatally to diethylstilbestrol. *Science* **190** (1975) 991–992.
17. Arai Y.: Metaplasia in male reproductive accessory glands induced by neonatal estrogen treatment. *Experientia* **24** (1968) 180–181.
18. McLachlan J. A.: Prenatal exposure to diethylstilbestrol in mice: toxicological studies. *J. Toxic. Environ. Hlth* **2** (1977) 527–537.
19. Gill W. B., Schumacher G. F. B. and Bibbo M.: Pathological semen and anatomical abnormalities in the genital tract in human male subjects exposed to diethylstilbestrol in utero. *J. Urol.* **117** (1977) 477.
20. Depue R. H., Pike M. C. and Henderson B. E.: Estrogen exposure during gestation and risk of testicular cancer. *J. Natn. Cancer Inst.* **71** (1983) 1151–1155.
21. Newbold R. R., Carter D. B., Harris S. E. and McLachlan J. A.: Molecular differentiation of the mouse genital tract: altered protein synthesis following prenatal exposure to diethylstilbestrol. *Biol. Reprod.* **30** (1984) 459–470.
22. Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J.: Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193** (1951) 265–275.
23. Laemmli U. K.: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227** (1970) 680–685.
24. Cathala G., Savouret J. F., Mendez B., West B. L., Martial M. K. and Baxter J. A.: A method for isolation of translationally active ribonucleic acid. *DNA* **2** (1983) 329–335.
25. Aviv H. and Leder P.: Purification of biologically active globin messenger RNA by chromatography on oligothymidilic acid cellulose. *Proc. Natn. Acad. Sci. U.S.A.* **69** (1972) 1408–1412.
26. Young R. A. and Davies R. W.: Efficient isolation of genes by using antibody probes. *Proc. Natn. Acad. Sci. U.S.A.* **80** (1983) 1194–1198.
27. Maniatis T., Fritsch E. F. and Sambrock J.: *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor, New York (1982).
28. White B. A. and Bancroft F. C.: Cytoplasmic dot hybridization. *J. Biol. Chem.* **257** (1982) 8569–8572.
29. Jean-Faucher Ch., Berger M., De Turckheim M., Veyssière G. and Jean Cl.: Developmental patterns of plasma and testicular testosterone in mice from birth to adulthood. *Acta Endocr.* **89** (1978) 780–788.
30. Rajfer J. and Coffey D. S.: Effects of neonatal steroids on male sex tissues. *Invest. Urol.* **17** (1979) 3–8.
31. Chung L. W. K. and Ferland-Raymond G.: Differences among rat sex accessory glands in their neonatal androgen dependency. *Endocrinology* **97** (1975) 145–153.
32. Donjacour A. A. and Cunha G. R.: The effect of androgen deprivation on branching morphogenesis in the mouse prostate. *Dev. Biol.* **128** (1988) 1–14.
33. Cunha G. R., Fujii H., Neubauer B. L., Shannon J. M., Sawyer L. and Reese B. A.: Epithelial-mesenchymal interactions in prostatic development. I. Morphological observations of prostatic induction by urogenital sinus mesenchyme in epithelium of the adult rodent urinary bladder. *J. Cell Biol.* **96** (1983) 1662–1670.
34. Gaytan F., Bellido C., Aguilar R. and Lucena M. C.: Morphometric analysis of the rat ventral prostate and seminal vesicles during prepubertal development: effects of neonatal treatment with estrogen. *Biol. Reprod.* **35** (1986) 219–225.
35. Neubauer B. and Mawhinney M.: Actions of androgen and estrogen on guinea pig seminal vesicle epithelium and muscle. *Endocrinology* **108** (1981) 680–687.
36. Turner T., Edery M., Mills K. T. and Bern H. A.: Influence of neonatal diethylstilbestrol treatment on androgen and estrogen receptor levels in the mouse anterior prostate, ventral prostate and seminal vesicle. *J. Steroid Biochem.* **32** (1989) 559–564.
37. Inczefi-Gonda A. and Csaba G.: Prolonged influence of a single neonatal steroid (dexamethasone) treatment on thymotic steroid binding. *Exp. Clin. Endocr.* **85** (1985) 358–360.
38. Jean-Faucher Ch., Berger M., Gallon C., De Turckheim M., Veyssière G. and Jean Cl.: Imprinting of male sex tissues by neonatal endogenous androgens in mice. Molecular alterations following exposure to cyproterone acetate. *Horm. Res.* **24** (1986) 38–45.
39. Veyssière G., Gallon C., Berger M., Jean-Faucher Ch., De Turckheim M. and Jean Cl: Prolonged influence of a neonatal cyproterone acetate treatment on renal androgen binding in mice. *Acta Endocr.* **119** (1988) 263–268.
40. Aihara M., Kimura T. and Kato J.: Dynamics of the estrogen receptor in the uteri of mice treated neonatally with estrogen. *Endocrinology* **107** (1980) 224–230.
41. Bellido C., Gaytan F., Aguilar R., Pinilla L. and Aguilar E.: Prepubertal reproductive defects in neonatal estrogenized male rats. *Biol. Reprod.* **33** (1985) 381–387.

42. Suzuki N., Hosoya T., Higarashi T. and Muramatsu M.: Changes in the template-engaged and free RNA polymerase I activities in isolated nuclei from rat ventral prostates after treatment with testosterone and cycloheximide. *J. Biochem.* **95** (1984) 1389–1398.
43. Moore J. T., Norwitch M. E., Wieben E. D. and Veneziale C. M.: Expression of a secretory protein gene during androgen-induced cell growth. *J. Biol. Chem.* **259** (1984) 14750–14756.
44. Taragnat C., Berger M. and Jean Cl: Preliminary characterization, androgen-dependence and ontogeny of an abundant protein from mouse vas deferens. *J. Reprod. Fert.* **83** (1988) 835–842.
45. Normand T., Jean-Faucher Ch. and Jean Cl.: Developmental patterns of androgen-regulated proteins from mouse seminal vesicle. *Int. J. Androl.* **12** (1989) 219–230.
46. Taragnat C., Berger M. and Jean Cl: Tissue and species specificity of mouse ductus deferens protein. *J. Androl.* (In press).
47. Pentecost B. T., Newbold R. R., Teng C. T. and McLachlan J. A.: Prenatal exposure of male mice to diethylstilbestrol alters the expression of the lactotransferrin gene in seminal vesicles. *Molec. Endocr.* **2,** (1988) 1243–1248.
48. Weintraub H.: Assembly and propagation of repressed and derepressed chromosomal states. *Cell* **42** (1985) 705–711.
49. Benvenisty N. and Reshef L.: Developmental expression and modification of genes. *Biol. Neonate* **52** (1987) 61–69.