Biochemistry

 Volume 21, Number 15 July 20, 1982

Effect of Castration on the Synthesis of Seminal Vesicle Secretory Protein IV in the Rat†

Michael C. Ostrowski,‡ Malathi K. Kistler, and W. Stephen Kistler*

ABSTRACT: The effects of castration on the synthesis (accumulation) of a major seminal vesicle secretory protein (SVS IV) were examined in young adult rats. In vitro incorporation of labeled amino acids into SVS IV by minced tissue was monitored by immunological methods. Castration resulted in a large decrease in the differential synthesis of SVS IV. A significant decrease in the relative incorporation of isotope into SVS IV was evident within 3 days of castration, and by 4 weeks relative incorporation dropped some 30-fold. These changes took place in the presence of a large generalized decline in protein synthesis so that incorporation into SVS IV on an organ basis decreased by over 200-fold. SVS IV messenger RNA levels were estimated by RNA excess solution hybridization using a cloned cDNA probe. Relative message levels declined after castration in harmony with the declines in SVS IV synthesis. SVS IV mRNA was decreased by a relative factor of ~20 and an absolute factor of ~200 in long-term (40-day) castrates. Accordingly, the seminal vesicle conforms to the general pattern of steroid regulated systems in which hormone withdrawal leads to differential decreases in the steady-state pool size for specific mRNAs. The seminal vesicle is unusual, however, in that a prolonged period is required for maximum differential effects to occur.

The rodent seminal vesicle is a major male accessory sex gland located at the base of the bladder. Under androgenic stimulation it makes and secretes large amounts of a few bulk protein species that ultimately become constituents of semen (Price & Williams-Ashman, 1961). The principal seminal vesicle secretory proteins are thus attractive candidates for study of the mechanisms by which androgens and other possible hormonal agents regulate the synthesis of specific proteins in the male reproductive tract. In recent years several laboratories have begun analysis of this question in the rat (Toth & Manyai, 1968; Higgins et al., 1976; Higgins & Parker, 1980; Ostrowski et al., 1979; Mansson et al., 1979) and the guinea pig (Veneziale, 1977; Veneziale et al., 1977a,b). When unfractionated rat seminal vesicle secretion is analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis according to the protocol of Laemmli (1971), five major protein bands are resolved that we have designated by Roman numerals starting with the slowest and numbering in order of increasing mobility (Ostrowski et al., 1979). We have focused our attention on the band IV protein (SVS IV) because of its ease of purification and small size. SVS IV corresponds to protein S of Higgins et al. (1976). Recently its complete amino acid sequence (90 residues in all) was reported by Pan et al. (1980).

Higgins and associates have analyzed a number of the effects of castration on the rat seminal vesicle including the synthesis of SVS IV. These authors concluded that total protein synthesis by the seminal vesicle was decreased dramatically following castration but that there was little or no selective effect upon the major secreted proteins such as SVS IV. That is, while a generalized decrease in protein synthesis followed castration, a pronounced differential effect upon secretory protein synthesis was not observed during the period examined (Higgins et al., 1976; Higgins & Burchell, 1978). Analysis of the complexity pattern of seminal vesicle RNA by kinetic hybridization studies was also interpreted to support such a model for generalized regulation of protein synthesis in these organs (Higgins et al., 1978, 1979).

Our own previous work concerned the changes in synthesis of SVS IV accompanying development of the seminal vesicle in young rats (Kistler et al., 1981a). In this situation we demonstrated clearly that a large increase in the differential synthesis of SVS IV occurs in rats between 25 and 60 days of age. Translational assays indicated that a differential increase in SVS IV message occurred during development as well, but that this change was rather modest compared to the increase in SVS IV synthesis. These results were interpreted to indicate that elements of translational regulation of SVS IV synthesis may take place in young animals (Kistler et al., 1981a).

We have now addressed the change in SVS IV synthesis

† From the Department of Chemistry and Biochemistry Section of the School of Medicine, University of South Carolina, Columbia, South Carolina 29208. *Received October 26, 1981.* This work was supported in part by grants from the March of Dimes Birth Defects Foundation and the U.S. Public Health Service (HD 13472).

* Address correspondence to this author at the Department of Chemistry, University of South Carolina.

‡ Present address: Laboratory of Tumor Virus Genetics, National Institutes of Health, Bethesda, MD 20205.

0006-2960/82/0421-3525$01.25/0

seen in older animals following androgen deprivation by castration. Using highly specific immunological assay procedures, we observed a striking drop (some 30-fold) in the differential synthesis (accumulation) of SVS IV following castration. Assay of the levels of SVS IV messenger RNA by RNA excess hybridization showed that a significant differential drop in SVS IV message also occurred and that most, if not all, of the decline in SVS IV synthesis can be correlated with the decreased pool of SVS IV message. While our studies were in progress, Mansson et al. (1981) also noted a large differential drop in SVS IV messenger RNA levels following long-term castration.

Experimental Procedures

Animals. Sprague–Dawley rats of known age were obtained from Charles River Breeding Laboratories (Wilmington, MA) or from Harlan Sprague–Dawley (Indianapolis, IN) and were housed locally with free access to Purina lab chow and water. Bilateral castration was performed by a scrotal route under ether anaethesia.

In Vitro Protein Synthesis by Seminal Vesicle Minces. Animals were killed by cervical dislocation and the seminal vesicles carefully dissected away from the coagulating glands. Upon removal from the animal the vesicles were freed of secretion by manual expression and placed on ice. Tissue was then finely minced with iridectomy scissors, and 50-mg portions were amassed from one or more animals, depending upon seminal vesicle weights. Tissues were then suspended in 1 mL of Dulbecco's modified Eagle's medium lacking methionine in individual 10-mL conical flasks. [^{35}S]Methionine (10 μCi) was added, and the stoppered flasks were incubated at 37 °C in an air atmosphere.

At desired points the flasks were transferred to ice, and the contents were mixed with 1 mL of 0.5 M sucrose, 8 mM $MgCl_2$, and 50 mM Tris-HCl,[1] pH 7.6, and then homogenized by using a Polytron (Brinkmann Instruments, Westbury, NY) at medium speed for 10 s. Portions of the combined medium–tissue homogenate were used to determine incorporation of radioactivity into cold 5% (w/v) trichloroacetic acid insoluble material using Whatman GFC glass fiber filter paper. Other portions were made 1% (w/v) in Triton X-100 and centrifuged (10 min, 17000*g*, 4 °C).

Immunological Isolation of SVS IV. Samples of the Triton-containing supernatant described immediately above were mixed in plastic vials with affinity purified anti-SVS IV covalently coupled to Sepharose 4B (Ostrowski et al., 1979) (1 mL of packed beads; capacity = 100 μg of SVS IV) and 1 mL of buffer containing 1.5% (w/v) Triton X-100, 1.5% (w/v) sodium deoxycholate, 0.15 M NaCl, 10 mM sodium phosphate, pH 7.0, and 0.02% (w/v) sodium azide. After being gently mixed at 4 °C for 4 h, the contents were transferred to small plastic columns and washed first with 25 mL of the above buffer and then with 25 mL of buffer lacking detergents. SVS IV was then eluted by 4 mL of formic acid [1 to 40 dilution by volume of 88% (w/w) stock]. Pure SVS IV (5 μg) was added as carrier to the eluate, which was lyophilized and then electrophoresed on sodium dodecyl sulfate gels. After being stained, the region containing SVS IV was excised, dissolved in 60% (w/w) H_2O_2 (5 h at 60 °C), and counted in a toluene/Triton X-100 based scintillation cocktail (Kistler et al., 1973). Recovery of SVS IV by this procedure has been shown to be 75–85% and to be proportional to the volume of tissue homogenate applied to the immobilized antibody (Kistler et al., 1981a).

Polyacrylamide Gel Electrophoresis. The sodium dodecyl sulfate system of Laemmli (1971) was followed by using slabs (0.15 cm thick × 8.5 cm long) for the separating gel with a 1-cm stacking gel. A gradient of 7–17% or sometimes 12–18% polyacrylamide was constructed by appropriate dilutions from a stock solution of 30% acrylamide and 0.27% methylenebis(acrylamide). The sample buffer solution of Laemmli was modified to contain 1% detergent and twice the Tris concentration specified. For fluorography, gels were dipped for 1 h in 2% (w/v) β-methylnaphthalene, 0.4% (w/v) 2,5-diphenyloxazole dissolved in glacial acetic acid, and then in water for 1 h. They were then dried under vacuum and exposed to Kodak X-Omat AR film at −70 °C.

Extraction of RNA. Total nucleic acid was prepared from 500 mg of freshly dissected tissue by homogenization in 20 mL of 1% sodium dodecyl sulfate, 10 mM Tris-HCl, pH 7.6, and 5 mM EDTA and the addition of proteinase K (500 μg) essentially as described by Lee et al. (1978). Recovered nucleic acid was dissolved in 2 mL of 25 mM NaCl, 10 mM Tris-HCl, pH 7.5, 10 mM magnesium acetate, and 2 mM $CaCl_2$ and treated with DNase I (RNase free; Worthington Biochemicals) at a concentration of 50 μg/mL for 30 min at 37 °C. The mixture was adjusted to 100 mM NaCl, 20 mM EDTA, and 0.1% sodium dodecyl sulfate to stop the reaction, and extraction with phenol–chloroform (1:1 v/v) was performed twice. After addition of sodium acetate to make 0.2 M, the RNA was precipitated with 2 volumes of absolute ethanol at −20 °C. In some cases it was necessary to repeat the DNase treatment. Any DNA contamination remaining was determined by the diphenylamine reaction (Burton, 1968), and RNA estimations by A_{260} measurements were adjusted accordingly.

Estimation of SVS IV Messenger RNA by RNA Excess Hybridization. Hybridization mixtures (50 μL) consisted of 50% (v/v) formamide (Fluka, treated with a mixed bed ion exchange resin), 0.5 M NaCl, 20 mM Hepes, pH 6.8, 0.1% sodium dodecyl sulfate, 0.5 mM EDTA, 100 μg/mL denatured calf thymus DNA, 40000 cpm/mL nick-translated probe (3.5 × 10^7 cpm/μg), and RNA varying from 3.4 ng to 1.088 mg/mL. Samples in 1.5-mL polypropylene tubes were overlaid with parafin oil, heated at 80 °C for 3 min, and then incubated at 43 °C for 24 h.

The reactions were chilled and mixed with 0.8 mL of S1 digestion buffer (300 mM NaCl, 30 mM sodium acetate, pH 4.5, 2 mM $ZnCl_2$). Half of each sample was then treated with S1 nuclease (P-L Biochemicals, 40 units) in the presence of 3.5 μg of denatured calf thymus DNA for 1 h at 37 °C. Both treated and untreated samples were then precipitated by the addition of 20 μL of DNA (3.5 mg/mL) and 500 μL of trichloroacetic acid (200 mg/mL). Precipitates were collected on glass-fiber filters (Whatman GF/C), and radioactivity was determined by using a nonaqueous scintillation fluid (4 g of PPO, 200 mg of POPOP, 1 L of toluene). Percent cDNA hybridized was taken from the ratio of trichloroacetic acid precipitable S1 resistant material to total acid precipitable counts for each hybridization reaction.

The probe used for hybridization was the SVS IV cDNA insert from recombinant plasmid pSVM 401 (Kistler et al., 1981b). The insert was excised by *Pst*1 digestion and isolated by preparative electrophoresis. Labeling was accomplished

[1] Abbreviations: Hepes, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; R_0t, (concentration of RNA as moles of nucleotides per liter)(seconds); Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; PPO, 2,5-diphenyloxazole; POPOP, 1,4-bis(5-phenyloxazol-2-yl)benzene.

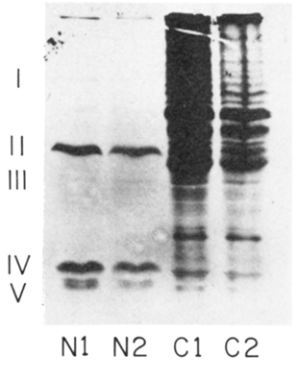

FIGURE 1: Fluorogram of proteins synthesized in vitro by seminal vesicle minces from intact and castrate rats. Rats were 104 days of age, and the experimental animals had been castrated 40 days previously. In vitro incubation was as described under Experimental Procedures. Total trichloroacetic acid insoluble material was obtained by chilling the tissue suspension on ice and by addition of 2 mL of distilled water and then 3 mL of 10% trichloroacetic acid followed by homogenization with a Polytron. The precipitate was collected by centrifugation (12000*g* × 15 min), washed 4 times with 10% trichloroacetic acid, twice with ethanol/ether (1:1 v/v) and once with ether, and dried at 40 °C. The dry material was then extracted with 0.4 mL of sample buffer for dodecyl sulfate electrophoresis (4 min at 100 °C followed by 16 h at 4 °C). Insoluble material was removed by centrifugation, and samples of 50 μL were then subject to electrophoresis on a 12–18% acrylamide gradient gel slab (120 V × 3.5 h). Each gel lane contains material resulting from extraction of 6 mg of tissue obtained from separate animals, either normal (N1 and N2) or castrate (C1 and C2). Counts per minute (cpm) applied to the gel were the following: N1, 28 000; N2, 21 000; C1, 44 000; C2, 40 000. The fluorogram was exposed 4 days at −70 °C. The locations of the major secretory proteins are marked.

by nick translation (Maniatis et al., 1975) with [α-^{32}P]dCTP (New England Nuclear).

Results

Effect of Castration on Proteins Synthesized by the Seminal Vesicle. If castration leads to a readily apparent change in the relative mix of proteins synthesized by the seminal vesicle, this ought to be evident from examination of the pattern of incorporation of [^{35}S]methionine into different proteins by vesicle tissue taken from intact and long-term castrate animals. Rats were castrated at 64 days of age, and 40 days later seminal vesicles from castrates and controls were removed, minced, and allowed to incorporate [^{35}S]methionine for 3 h under in vitro conditions as described under Experimental Procedures. Separation of total 1% dodecyl sulfate soluble proteins by polyacrylamide gel electrophoresis (Figure 1) indicated that a very different population of proteins were labeled by tissues from intact and castrate animals. With control animals, the major secretory proteins totally dominated the pattern of radioactive proteins resolved. In striking contrast, the proteins labeled by tissue from the 40-day castrates showed only faint bands that might correspond to the secretory proteins despite the ready labeling of a host of other protein species. Longer exposure of this gel showed that many of the protein bands labeled by castrate tissues are also synthesized in normal animals, though as a much diminished proportion of total protein (results not shown). These results caused us to proceed with a more detailed study of the synthesis of the major secretory protein SVS IV in castrate animals.

Changes in Seminal Vesicle Weight and Secretory Activity following Castration. Two groups of animals were castrasted, one at 60 days of age and the second at 120 days of age. The younger animals actively synthesize SVS IV and other secretory proteins, though the seminal vesicles have not yet reached their full adult weight (Kistler et al., 1981a). The

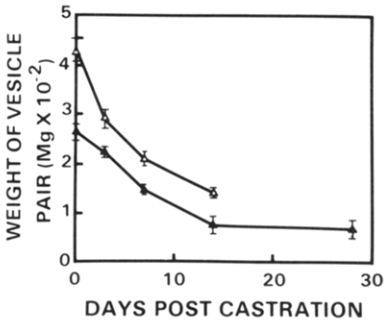

FIGURE 2: Seminal vesicle weight at periods following castration. Weights refer to pairs of organs following manual expression of any secretion present. Animals were 60 days old (▲) or 120 days old (△) at time of castration. Bars indicate range of weights observed.

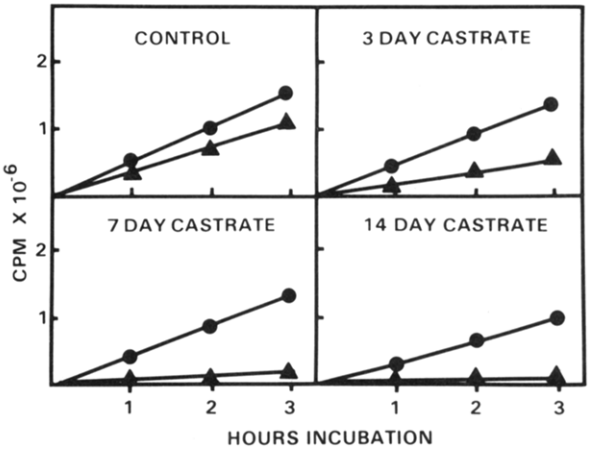

FIGURE 3: In vitro incorporation of [^{35}S]methionine by minced seminal vesicle into 5% trichloroacetic acid insoluble material. Incorporation into total tissue plus (●) medium or into medium alone (▲) was determined as described under Experimental Procedures. Incorporation is expressed per 50 mg of tissue.

120-day-old rats are fully sexually mature. Following castration, seminal vesicle weights fell significantly for both groups of animals (Figure 2). Over the 2-week period following castration of 60-day-old rats, equivalent weights of tissue from intact and castrate rats incorporated approximately the same amount of label into total acid insoluble material (Figure 3). Tissue from intact animals released (secreted) about two-thirds of the labeled material onto the medium during the incubation. Following castration, however, labeled material appearing in the medium fell to a negligible value. Similar results were obtained with tissue from animals castrated at 120 days of age (results not shown).

Effect of Castration on Incorporation of [^{35}S]Methionine into SVS IV. The percentage of incorporation of label into SVS IV was determined by using a two-step isolation procedure involving, first, a highly specific immobilized antibody to SVS IV and, second, electrophoretic purification of the material retained by the antibody preparation. This recovery system was carefully standardized to ensure that there was adequate capacity present for the amount of tissue homogenate used and that labeled standard SVS IV could be recovered in the presence of tissue extracts in a reproducible fashion (Kistler et al., 1981a). With animals castrated at 60 days of age, a pronounced fall in the incorporation of [^{35}S]methionine into total acid insoluble material was evident at 3 days following castration, and by 4 weeks, incorporation on a gland basis was decreased approximately 9-fold (Figure 4A). Over the same period the proportion of radioactivity incorporated into SVS IV also declined with approximately parallel kinetics. Percentage incorporation into SVS IV fell by at least 30-fold

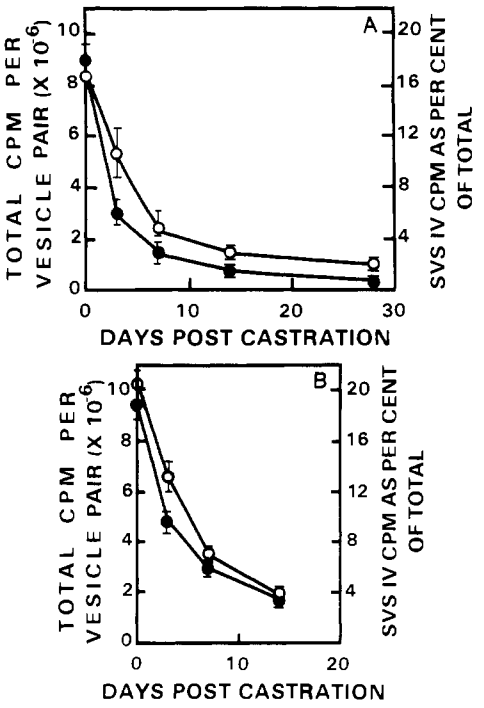

FIGURE 4: In vitro incorporation of [^{35}S]methionine into total trichloroacetic acid insoluble material or into SVS IV by tissue from animals castrated at 60 (A) or 120 (B) days of age. Incorporation into total protein (○) is expressed on an organ basis. Incorporation into SVS IV (●) is expressed as a *percent* of incorporation into total acid insoluble material. Points are the averages of two flasks while the bars represent the range of values. Values are taken from 3-h incubations. For details, see Experimental Procedures.

Table I: Changes in Incorporation of [^{35}S]Methionine into SVS IV on an Organ Basis following Castration

days postcastration	castrate at 60 days		castrate at 120 days	
	cpm/ organ pair[a]	%	cpm/ organ pair[a]	%
0 (intact)	1.51	100	1.94	100
3	0.323	21	0.63	32
7	0.072	4.8	0.32	16
14	0.022	1.5	0.067	3.5
28	0.0062	0.41		

[a] cpm × 10^{-6}. Data are taken from Figure 4A,B.

following 4 weeks of castration (Figure 4A). Expressed as absolute incorporation of radioactivity on an organ basis (Table I) SVS IV synthesis fell by nearly 250-fold during the experimental period.

Comparable results were obtained when 120-day-old rats were castrated, though the fall in percent incorporation into SVS IV was somewhat more gradual with the older animals (Figure 4B and Table I).

Change in SVS IV Messenger RNA following Castration. RNA excess solution hybridization was used to compare relative SVS IV mRNA levels in control animals with 14- and 40-day castrates (Figure 5). The hybridization curves were displaced by factors of approximately 6 and 20, respectively, for the castrate animals, indicating significant declines in the relative proportion of SVS IV sequences. When the 10-fold decrease in total RNA resulting from castration (Figure 5 legend) is considered, the absolute level of SVS IV message dropped by a factor of 200 in the 40-day castrates. While the decreases measured in the steady-state pool for SVS IV mRNA were not quite as great as the decrease of SVS IV synthesis, they clearly account for most, if not all, of that decline.

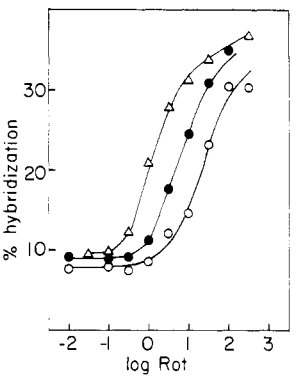

FIGURE 5: Hybridization kinetics of reactions between pSVM 401 insert and total seminal vesicle RNA preparations from intact and castrate animals. RNA samples were from intact (△), 4-day castrate (●), or 40-day castrate (○) animals. RNA samples were obtained from pooled tissues as follows: intact animals, 2 rats, recovery of 873 μg/animal; 14-day castrates, 6 rats, 86 μg/animal; 40-day castrates, 8 rats, 80 μg/animal. No corrections have been applied to the hybridization data. The probe in the absence of added RNA showed a zero time hybridization amounting to 6–7%, which increased to about 9–10% following 24 h of incubation.

Discussion

In this investigation we have addressed the question of whether deprivation of testicular androgens by castration results in substantial differential effects on the synthesis (accumulation) of one of the major secretory proteins of the rat seminal vesicle. Using immunological isolation procedures, we have shown that castration does indeed lead to a large differential decrease in the ability of seminal vesicle tissue to incorporate labeled amino acids into SVS IV. Incorporation decreased by a relative factor of about 30 in the face of a roughly 9-fold drop in total protein synthesis with tissue from long-term (30-day) castrates. Qualitatively similar results were obtained with animals castrated at either 60 or 120 days of age, though the rate of decline of incorporation into SVS IV was somewhat slower with the older animals. This differential loss in the capacity to produce SVS IV following castration is compatible with our previous study showing that a marked *increase* in the differential incorporation into SVS IV accompanied the sexual development of the seminal vesicles in pubertal rats (Kistler et al., 1981a). Our observations of differential changes in the synthesis of a major secretory protein contrast with those of Higgins and associates in the rat (Higgins et al., 1976; Higgins & Burchell, 1978) and of Veneziale et al. (1977b) in the guinea pig, who reported large absolute but only slight relative decreases in secretory protein synthesis following castration. In the case of the guinea pig, the discrepancy may relate only to the differences between relatively short-term castration (5 days) and longer experimental periods.

Measurements of the steady-state pool size of SVS IV messenger RNA indicated that declines in message content were roughly proportionate to falls in SVS IV synthesis. Accordingly, SVS IV synthesis in long-term castrates is seemingly regulated chiefly at the level of mRNA pool size. Our results agree qualitatively with those of Mansson et al. (1981) who noted even more striking differential decreases in SVS IV mRNA levels with their experimental animals. These authors demonstrated that testosterone administration to long-term castrate animals resulted in rapid induction of SVS IV message, thus showing unambiguously the major role of androgens in this system. During sexual development of the seminal vesicles, a discrepancy between the low proportion of total protein synthesis devoted to SVS IV in immature animals

and the relatively high proportion of SVS IV in RNA found among translatable messages suggested some degree of selective translational control over the SVS IV synthesis (Kistler et al., 1981a). Since in the current study message levels in 14-day and longer castrate animals correlated well with the relative levels of SVS IV synthesis, translational effects, if they occur, are evidently of minor importance. However, the possible role of such translational effects in the early period of castration deserves additional attention.

While the pattern of regulation seen in the seminal vesicles is similar to that reported with other steroid regulated systems, these glands respond very slowly to hormonal deprivation in comparison with other organs. For example, withdrawal of estrogen from immature chicks leads to at least a 1000-fold drop in the relative concentration of ovalbumin message (Harris et al., 1975; McKnight et al., 1975), with most of this decrease occurring in the first day without estrogen (Cox, 1977; Hynes et al., 1979). Similarly, by 7 days following castration the relative concentration of mRNA for a prominent steroid binding protein of the rat prostate (Fang & Liao, 1971; Heyns et al., 1977; Parker & Scrace, 1979) falls by 3 orders of magnitude (Parker et al., 1980).

The reason for the slow change in the composition of the seminal vesicle mRNA pool following castration is unknown. While histological involution of the organs is essentially complete within 2 weeks following castration as observed at either the light (Moore et al., 1930) or electron microscope level (Dahl & Tveter, 1973), the proportion of total protein synthesis devoted to SVS IV at that point is still high compared to what it will be following a month of castration. Perhaps the hormonal milieu remaining in castrate animals continues to exert some positive differential effects on the pattern of mRNA accumulation. For example, low level androgens of adrenal origin might play a role. Prolactin has been implicated from time to time in the maintenance of rat seminal vesicle (Döhler & Wottke, 1975; Negro-Vilar et al., 1977), although Atassi et al. (1981) found no evidence that prolactin was required to restore seminal vesicle function in hypophysectomized guinea pigs. In view of the dramatic effect of prolactin on the stabilization of casein messenger RNA in the rat mammary gland (Rosen et al., 1980), its possible effects on the levels of specific messenger RNAs in the seminal vesicle deserve to be considered.

Acknowledgments

We are grateful to Robert Taylor for adept assistance in the handling of recombinant DNA molecules.

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