Influence of incubation conditions, tunicamycin and castration on incorporation of [³H]mannose and [³H]fucose into rat epididymal glycoproteins *in vitro*

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Summary. The incorporation of radioactive mannose and fucose into secretory glycoproteins by rat epididymal tissue was studied using tissue pieces *in vitro*. The appearance of radioactive macromolecular products in the medium occurred after a lag phase of 2 h with radioactive mannose, but with radioactive fucose the lag phase was only 15 min. Preincubation of tissue for 2 h before the addition of radioactive mannose increased the subsequent rate of incorporation by reducing the lag phase from 2 to 1 h. Tunicamycin reduced the incorporation of radioactive mannose and fucose into macromolecular products to approximately 15 and 50% of normal in the caput and cauda respectively; maximum inhibition required 10 µg tunicamycin/ml in the caput and 2 µg/ml in the cauda. Reduction of radioactive sugar incorporation by tunicamycin did not result in qualitative changes in the profile of the radioactive glycoproteins that were secreted. However, immunoprecipitation of proteins D and E from incubations with radioactive methionine or mannose revealed that tunicamycin caused these proteins to be synthesized and secreted in a non-glycosylated form. Prior castration of animals reduced the incorporation of radioactive mannose and fucose, and qualitative changes in the profiles of secreted radioactive glycoproteins were apparent.

Introduction

The epididymis synthesizes and secretes a number of glycoproteins (Brooks & Higgins, 1980; Jones, Brown, von Glos & Parker, 1980; Brooks, 1981a), some of which become bound to spermatozoa (Lea, Petrusz & French, 1978; Kohane, Garberi, Cameo & Blaquier, 1979; Kohane, Cameo, Pineiro, Garberi & Blaquier, 1980a; Kohane, Gonzalez Echeveria, Pineiro & Blaquier, 1980b; Faye, Duguet, Mazzuca & Bayard, 1980; Bayard, Duguet, Mazzuca & Faye, 1981; Jones, Pholpramool, Setchell & Brown, 1981). The binding of glycoproteins may be responsible for an alteration in the surface characteristics of spermatozoa, now thought to be an integral component of sperm maturation. This refers to the process whereby spermatozoa develop the ability to fertilize as they pass through the epididymal duct. Hence, careful characterization of epididymal glycoproteins and knowledge of the regulation of their synthesis and secretion is of fundamental importance to understanding the factors regulating male fertility and sterility.

Several radioactive sugars are incorporated by epididymal tissue *in vitro* into a variety of secreted glycoproteins (Brooks, 1981a) although the synthesis of these secreted glycoproteins is not uniform; pronounced regional differences are apparent both in the overall rate of synthesis and in the types of secreted protein (Brooks, 1981a). Regional differences have been demonstrated in the content of dolichol, a cofactor in the synthesis of *N*-linked oligosaccharide chains in glycoproteins

(Lennarz, 1975; Parodi & Leloir, 1979), in the epididymis and in its rate of synthesis (Wenstrom & Hamilton, 1980). As the epididymis contains the greatest concentration of this polyprenol in any tissue studied so far (Wenstrom & Hamilton, 1980), it might be inferred that epididymal tissue is particularly active in the synthesis of glycoproteins containing *N*-linked oligosaccharides. Oligosaccharides linked in this fashion are usually characterized by a high content of mannose, whereas O-linked oligosaccharides are generally devoid of this sugar (Schachter, 1978). Hence mannose would appear to be a particularly useful precursor for studying the synthesis of *N*-linked glycoproteins in the epididymis. Fucose, which occurs in *N*-linked and O-linked oligosaccharides, has also been used as a precursor for glycoprotein synthesis. This sugar has the advantage that it is not converted to other derivatives (Bekesi & Winzler, 1967).

Tunicamycin is a specific inhibitor of the dolichol-mediated transfer of oligosaccharides to glycoproteins (Tkacz & Lampen, 1975). However, this inhibitor has little effect on the profile of radioactive glycoproteins secreted by epididymal tissue incubated with radioactive mannose (Brooks, 1981a). It was decided, therefore, to examine the characteristics of mannose incorporation into epididymal glycoproteins, and to establish the best conditions under which to demonstrate any effects of tunicamycin on epididymal glycoprotein synthesis. Because androgens regulate many epididymal functions (Brooks, 1981b), the influence of androgen status on epididymal glycoprotein synthesis was also studied.

Materials and Methods

Chemicals and animals

Details of the source of most chemicals are given by Brooks (1981a): D-[2-³H]mannose (sp. act. 16 Ci/mmol), L-[1-³H]fucose (sp. act. 5.4 Ci/mmol), L-[³⁵S]methionine (sp. act. 1385 Ci/mmol) and ¹⁴C-labelled L-amino acid mixture (code CFB. 104, mixture of amino acids in the proportions of an algal protein hydrolysate) were from Amersham Australia Pty Ltd, Sydney, Australia. Tunicamycin was a gift from Lilly Industries Pty Ltd, Sydney, Australia. A stock solution of 1 mg/ml was prepared in 25 mM-NaOH. IgG-sorb, a preparation of killed *Staphylococcus aureus*, was obtained from The Enzyme Center, Inc., Boston, Massachusetts, U.S.A. Adult male Sprague-Dawley rats were housed at 25°C with free access to food and water and a lighting regimen of 12 h light:12 h dark. Castration was performed under ether anaesthesia by the abdominal route (Brooks, 1976).

Incubation conditions

Animals were killed by decapitation. After the removal of excess adipose tissue the epididymis was subdivided into 10 arbitrary segments (see Pl. 1, Fig. 1a; Brooks, 1981a). Selected segments were chopped finely, washed in Ringer and 25 mg aliquants were incubated at 32°C in 1 ml Ringer in siliconized glass vials as described previously (Brooks, 1981a). Radioactive precursors and other components were added as indicated in the text. At the end of the incubation, the medium was carefully removed from the tissue pieces and centrifuged at 100 000 *g* for 30 min at 2°C. Alternatively 2.5 ml 0.5 M-sucrose, 3 mM-MgCl₂, 20 mM-Tris-HCl, pH 7.4, and 1.5 ml water were added to the total contents of the incubation vial. The contents were homogenized in a Potter-Elvehjem homogenizer fitted with a Teflon pestle and then centrifuged at 100 000 *g* for 30 min at 2°C. The extent of incorporation of radioactive precursor into macromolecular products was determined by filter-counting as described previously (Brooks & Higgins, 1980; Brooks, 1981a).

Comparisons between treatments were always made using tissue aliquants prepared within the same experiment. This was considered necessary since the pronounced differences between epididymal regions, and possibly between animals, could otherwise lead to erroneous conclusions.

Polyacrylamide gel electrophoresis

Electrophoresis was performed under denaturing conditions using the buffer system of Laemmli (1970) and a gradient of 9–13% acrylamide in the resolving gel (see Brooks, 1981a). Samples were prepared for electrophoresis as described by Brooks & Higgins (1980). Briefly, this involved precipitation with trichloroacetic acid, washing with ethanol:ether (1:1, v/v) and dissolving in dispersion buffer (30 mM-Tris-HCl, pH 6.8, 1% SDS, 10% glycerol, 0.002% bromophenol blue) with or without 5% 2-mercaptoethanol. The migration position of radioactive proteins was determined by fluorography (Bonner & Laskey, 1974) using pre-flashed X-ray film (Laskey & Mills, 1975).

Immunoprecipitation of specific epididymal proteins

Two rabbit antisera were used. One antiserum which recognized common antigenic determinants on proteins B and C (mol. wts 16 000) was prepared as described by Brooks (1981a). The other antiserum recognized common antigenic determinants on glycoproteins D and E (mol. wt 27 000 and 28 000), respectively (Brooks, 1982). Immune precipitation of radioactive proteins with IgG-sorb before polyacrylamide gel electrophoresis was carried out by the procedure of Ivarie & Jones (1979). Reagent volumes were selected to ensure quantitative precipitation of antigen. A control in which preimmune serum replaced immune serum was always included.

The amount of radioactivity present in bands located on polyacrylamide gels by fluorography was quantified by cutting out appropriate segments from the dried gel. These were digested in 1 ml H_2O_2 at 55°C for 48 h. Scintillation fluid (toluene:triton X-100 (2:1, v/v)) containing 2.6 g 2,5-diphenyloxazole and 0.2 g 1,4-bis-(5-diphenyloxazol-2-yl)benzene/l, 10 ml, was added before radioactivity counting.

Results

Characteristics of radioactive sugar incorporation

The incorporation of radioactive mannose into total acid-insoluble products by epididymal tissue was not linear (Text-fig. 1a). Very little mannose was incorporated during the first hour, and during each succeeding hour incorporation exceeded that in the previous hour. Radioactive proteins did not appear in the medium until 2 h, about 1 h after incorporation had begun to increase within the tissue (Text-fig. 1a). Only a small lag was observed when [^3H]fucose was used as the radioactive precursor (Text-fig. 1c).

Preincubation of tissue before the addition of radioactive mannose resulted in greater rates of mannose incorporation (Text-fig. 2); between 1 and 2 h of preincubation were required for maximum rates of incorporation. Preincubation reduced the lag observed in mannose incorporation, with radioactive proteins appearing in the medium after 1 h (Text-fig. 1b).

When the amount of radioactive mannose was kept constant, but increasing amounts of non-radioactive mannose were added to the medium, incorporation of radioactivity was not substantially reduced until the concentration of mannose exceeded about 0.1 mM (Text-fig. 3). Small changes in mannose concentration when [^3H]mannose was added without carrier evidently had negligible influence on the extent to which radioactivity was incorporated, but presumably overall incorporation of mannose (radioactive plus non-radioactive) increased with higher mannose concentrations.

Preincubation substantially increased the incorporation of [^3H]mannose in the initial segment (segment 1) and in segments 3 and 4 of the caput (compare b and c in Pl. 1, Fig. 1), but incorporation in the remainder of the epididymis was little affected. Preincubation did not cause qualitative changes in the profile of secreted radioactive proteins except for segment 1 (compare d

Text-fig. 1. Time course of incorporation of $[^3\text{H}]$ mannose and $[^3\text{H}]$ fucose into acid-insoluble products by epididymal tissue. Tissue pieces from the caput epididymidis were incubated with radioactive precursor: (a) segments 3 and 4 with $10 \mu\text{Ci}$ ($1 \mu\text{M}$) $[^3\text{H}]$ mannose; (b) segments 3 and 4 with $10 \mu\text{Ci}$ ($1 \mu\text{M}$) $[^3\text{H}]$ mannose added after 2 h preincubation in the absence of radioactive precursor; (c) segment 4 with $5 \mu\text{Ci}$ ($0.9 \mu\text{M}$) $[^3\text{H}]$ fucose. Radioactivity measured in samples at zero time was subtracted from all results. ●, Radioactivity in tissue plus medium; ▲, radioactivity in medium. Values are mean $\pm$ s.e.m. of 4 determinations.

Text-fig. 2. Effect of the duration of preincubation of epididymal tissue without mannose on the subsequent incorporation of $[^3\text{H}]$ mannose into acid-insoluble products in tissue plus medium. Tissue pieces from the caput epididymidis (segments 3 & 4) were preincubated without radioactive precursor. $[^3\text{H}]$ Mannose ($10 \mu\text{Ci}$, $0.6 \mu\text{M}$) was added at various times after the start of the preincubation and the incubation was then continued for a further 3 h. Values are mean $\pm$ s.e.m. of 3 determinations.

and e in Pl. 1, Fig. 1): apparent qualitative differences in caput segments resulted from intensity differences due to the 3-fold difference in the amount of radioactive protein.

Effect of tunicamycin on radioactive sugar incorporation

Tunicamycin reduced mannose incorporation when added without preincubation, but maximum inhibition was observed when tissue was preincubated with tunicamycin for 2 h before the addition of radioactive mannose (Text-fig. 4).

PLATE 1

Fig. 1. Effect of preincubation on the extent of $[^3\text{H}]$ mannose incorporation and on the profile of radioactive glycoproteins secreted by various segments (a) of the epididymis. Two aliquants (25 mg) of washed tissue pieces from each segment were incubated in Ringer: one was incubated directly with $50 \mu\text{Ci}$ ($5 \mu\text{M}$) $[^3\text{H}]$ mannose (b, control) for 3 h and the other was preincubated for 2.5 h before the addition of $[^3\text{H}]$ mannose and further culture for 3 h (c). Polyacrylamide gel electrophoresis was run under non-reducing conditions (d, control; e, preincubated) and each lane contained the amount of radioactive protein indicated in (b) and (c) respectively. The arrow in (d) indicates the band of 70 000 mol. wt (see text).

PLATE 2

Fig. 2

Fig. 3

PLATE 3

Fig. 4. Effect of castration on the profile of radioactive proteins synthesized and secreted by epididymal tissue. Tissue pieces from the caput (segment 4) and cauda (segment 8) of normal animals or animals castrated 1 month previously were incubated with [^3H]fucose (normal tissue, 50 μCi ; 'castrate' tissue, 100 μCi) or [^3H]mannose (normal tissue, 100 μCi ; 'castrate' tissue, 300 μCi) for 5 h. An equal amount (12 000 c.p.m.) of radioactive protein present in the medium at the end of the incubation was separated by gel electrophoresis and visualized by fluorography. Incubations with [^3H]fucose are in lanes 1–4 and those with [^3H]mannose in lanes 5–8. Lanes 1 and 5, normal caput; lanes 2 and 6, 'castrate' caput; lanes 3 and 7, normal cauda; lanes 4 and 8, 'castrate' cauda. Arrows indicate the bands of 40 000 and 28 000 referred to in the text.

Text-fig. 3. Effect of mannose concentration on the incorporation by epididymal tissue of [^3H]mannose into acid-insoluble products in tissue plus medium. Tissue pieces from the caput epididymidis (segments 3 & 4) were preincubated for 2 h. Then each incubation flask received 10 μCi (0.6 μM) of [^3H]mannose plus various concentrations of non-radioactive mannose, and the incubation was continued for 3 h. Values are mean $\pm$ s.e.m. of 3 determinations.

Near maximum inhibition of mannose incorporation in cauda tissue was achieved with 2 μg tunicamycin/ml, but much greater amounts of tunicamycin (10 $\mu\text{g}/\text{ml}$) were required to achieve maximum inhibition in the caput (Text-fig. 5). The extent of inhibition by tunicamycin was much greater in the caput than in the cauda (85 and 50%, respectively). This large reduction in mannose incorporation was not due to any great reduction in protein synthesis, which fell by only 30% at concentrations up to 20 μg tunicamycin/ml (Text-fig. 5).

PLATE 2

Fig. 2. Effect of tunicamycin on the profile of radioactive proteins synthesized and secreted by epididymal tissue. Tissue pieces from the caput (segment 4) or cauda (segment 8 for mannose, segment 9 for fucose) were incubated with tunicamycin (10 and 2 $\mu\text{g}/\text{ml}$, respectively). After 2 h, radioactive mannose or fucose was added and the incubation was continued for 3 h. The medium was separated from the tissue and processed for polyacrylamide gel electrophoresis. Equal amounts of radioactive protein from control and tunicamycin-treated incubation media were separated on the gels and radioactive bands were visualized by fluorography. The presence or absence of tunicamycin is indicated by the + or - above the lane. Lanes 1 and 2, caput with [^3H]mannose (control, 50 μCi ; tunicamycin, 250 μCi); lanes 3 and 4, caput with [^3H]fucose, (control, 100 μCi ; tunicamycin, 100 μCi); lanes 5 and 6, cauda with [^3H]mannose (control, 166 μCi , tunicamycin, 333 μCi); lanes 7 and 8, cauda with [^3H]fucose (control, 100 μCi , tunicamycin, 100 μCi).

Fig. 3. Effect of tunicamycin on secretion of specific proteins by epididymal tissue. Tissue pieces from the caput (segment 4) and cauda (segment 8) were incubated with or without tunicamycin (10 $\mu\text{g}/\text{ml}$, caput; 2 $\mu\text{g}/\text{ml}$, cauda) for 2 h. [^{35}S]Methionine (50 μCi) or [^3H]mannose (control, 100 μCi ; tunicamycin, 300 μCi) was then added and the incubation continued for 4 h. Either 100 000 c.p.m. radioactive protein from the medium ([^{35}S]methionine as precursor) or the total incubation medium ([^3H]mannose as precursor), were immunoprecipitated. The immunoprecipitates were separated by gel electrophoresis and radioactive bands visualized by fluorography. The presence or absence of tunicamycin is indicated by the + or - above the lane. Lanes 1-3, caput secretory proteins labelled with [^{35}S]methionine treated with antiserum against proteins B and C (lane 1, preimmune serum); lanes 4 and 5, caput secretory proteins labelled with [^3H]mannose treated with antiserum against proteins D and E; lanes 6-8, caput secretory proteins, and lanes 9 and 10, cauda secretory proteins, labelled with [^{35}S]methionine and treated with antiserum against proteins D and E (lane 6, preimmune serum). The migration positions of proteins B, C, D, D' and E' are indicated. The sum of the radioactivity present in all immunoprecipitated bands from tunicamycin incubations as a percentage of that in control incubations is shown by the figures in parentheses at the bottom of the gel lanes.

Text-fig. 4. Effect of the duration of preincubation of epididymal tissue with tunicamycin on the subsequent incorporation of [^3H]mannose into acid-insoluble products in tissue plus medium. Tissue pieces from the caput epididymidis (segments 3 & 4) were incubated without (control) or with 10 μg tunicamycin/ml. [^3H]Mannose (10 μCi , 0.6 mM) was added at various times after the start of preincubation and the incubation was then continued for 3 h. Each point represents the average of 2 determinations.

Text-fig. 5. Effect of tunicamycin concentration on the incorporation by epididymal tissue of [^3H]mannose and [^{14}C]amino acids into acid-insoluble products in tissue plus medium. Aliquants (25 mg) of washed tissue pieces from the caput (●, ■; segments 3 & 4) or cauda (▲, segment 8) epididymidis were preincubated for 2 h with various concentrations of tunicamycin before the addition of 10 μCi (0.6 μM) [^3H]mannose (●, ▲) or 0.25 μCi ^{14}C -labelled amino acids (■) and incubation for 3 h. Each point represents the average of 2 determinations.

Despite the extensive inhibition of glycosylation by tunicamycin, particularly in the caput, the profile of secreted radioactive proteins resulting from [^3H]mannose or [^3H]fucose revealed only minor differences (Pl. 2, Fig. 2), suggesting that tunicamycin inhibits the glycosylation of all secreted glycoproteins to the same degree.

Immunoprecipitation of the non-glycosylated proteins B and C from incubations with [^{35}S]methionine revealed that tunicamycin had no effect on their secretion (Pl. 2, Fig. 3, lanes 1–3) but immunoprecipitation of glycoproteins D and E from the same incubation media revealed marked alterations in their migration positions on polyacrylamide gels (Pl. 2, Fig. 3, lanes 6–10). A very similar pattern was obtained by immunoprecipitation of soluble proteins recovered from the homogenized tissue pieces (results not shown), indicating that the pattern of these secreted proteins provides a good index of their synthesis.

In the caput, in the presence of tunicamycin, the band migrating as protein D was fainter than in the control whereas a band D' with lower apparent molecular weight became prominent (Pl. 2, Fig. 3, lane 8). The total radioactivity in these two bands was similar to that in the single band from the control incubation. Presumably the band D' of lower molecular weight represents the non-glycosylated form of protein D which is still recognized by the antiserum. This conclusion is supported by the fact that immunoprecipitation of secretory proteins labelled with [³H]mannose revealed a band in the position of D (glycosylated) with no evidence of a band at D' (non-glycosylated) (Pl. 2, Fig. 3, lanes 4 and 5).

The results for the cauda were similar, but more complex because this region of the epididymis synthesizes protein E in addition to protein D and both proteins are recognized by the antiserum. In the presence of tunicamycin, fainter bands were seen in the positions of D and E with additional bands at D' and E' migrating with lower molecular weight corresponding to putative non-glycosylated forms of proteins D and E respectively (Pl. 2, Fig. 3, lane 10).

Effect of castration on radioactive sugar incorporation

Comparison of castrated and normal animals with respect to the profile of radioactive proteins released into the medium following incubation with [³H]mannose or with [³H]fucose (Pl. 3, Fig. 4) revealed qualitative differences which contrasted with the non-qualitative effects of tunicamycin. Changes were particularly evident in the cauda (Pl. 3, Fig. 4). The caput of normal animals contained bands of 40 000 and 28 000 mol. wt which corresponded to bands with slightly slower migration in the caput of castrated animals (Pl. 3, Fig. 4). These 40 000 and 28 000 bands represented subunits of a larger protein of 70 000 mol. wt (Pl. 1, Fig. 1d) in which the subunits were held together by disulphide bonds under non-reducing conditions. The relationship between the 70 000 mol. wt protein and the 40 000 and 28 000 subunits was confirmed by cutting out the former band from a dried gel and re-running it on a second gel under reducing conditions (results not shown).

Discussion

It was found in a previous study that radioactive sugars are incorporated into secretory glycoproteins by epididymal tissue *in vitro*, but that the extent of incorporation and the types of secreted glycoproteins vary considerably between different epididymal segments (Brooks, 1981a). Before proceeding with further investigations of the regulation of glycoprotein synthesis and secretion by the epididymis, a more detailed study of the characteristics of the incubation system seemed warranted. Several new features were revealed in the present study. Most importantly, a distinct lag was found for the incorporation of [³H]mannose into macromolecular products; secreted radioactive glycoproteins were not found in the medium until 2 h after the start of the incubation. Possible explanations for this lag are that mannose transport into the cells suffers competition from glucose in the medium (Brooks, 1979) or that there is preferential utilization and depletion of an intracellular pool of some precursor(s) before exogenous mannose is able to enter the metabolic sequence. The latter interpretation is supported by the observation that preincubation without mannose greatly increased the subsequent incorporation of [³H]mannose by effectively halving the lag period.

The residual lag period of 1 h which still remained after preincubation is similar to that observed with [³⁵S]methionine as precursor (Brooks, 1981a) and corresponds well with the delay *in vivo* between the administration of a radioactive amino acid and the appearance of radioactive protein in the epididymal lumen (Flickinger, 1979, 1981). This would reflect the time taken for newly formed protein to pass in exocytotic vesicles from the cisternae of the endoplasmic reticulum to the cell surface by way of the Golgi apparatus.

The lag observed for the incorporation of [^3H]mannose was in direct contrast to the minimal lag for [^3H]fucose. Most probably this difference partly reflects a limited precursor pool of fucose or its derivatives within the tissue. However, the difference in lag times is also likely to be due to the different pathways involved in the incorporation of these precursors into glycoproteins. Mannose is present in the core of the *N*-linked oligosaccharide side chains which are first built up on a polyprenoid carrier (dolichol) before transfer to the protein during translation. Subsequently the core oligosaccharide is modified by the removal of some monosaccharide units and the addition of others (Staneloni & Leloir, 1979; Gibson, Kornfeld & Schlesinger, 1980; Hanover & Lennarz, 1981). Fucose, on the other hand, is usually added as a terminal unit to oligosaccharide side chains late in glycoprotein synthesis after the core oligosaccharide chain has been added to the protein and pruned of excess monosaccharide units (Waechter & Lennarz, 1976; Hanover & Lennarz, 1981).

Tunicamycin specifically inhibits dolichol-mediated synthesis of *N*-glycosidic linked oligosaccharides (Tkacz & Lampen, 1975). Such oligosaccharides typically have a high content of mannose, whereas *O*-glycosidic linked oligosaccharides are apparently devoid of mannose in mammalian systems (Schachter, 1978). Brooks (1981a) reported that tunicamycin reduced the incorporation of [^3H]mannose into secretory glycoproteins but did not alter the profile of secreted radioactive glycoproteins. In the present study, preincubation of tissue for ~ 2 h with tunicamycin was required for the maximum reduction in incorporation of radioactive precursor. Presumably preincubation allows endogenous reserves of dolichol derivatives to be depleted and enables the antibiotic to form its inhibitory complexes. The difference in behaviour of the caput and cauda tissues towards tunicamycin with respect to the concentration of tunicamycin required for maximum inhibition of glycosylation is quite consistent with the observations of Wenstrom & Hamilton (1980) that the caput contains twice the dolichol content of the cauda and is more than twice as active in the synthesis of dolichol from 2-[^{14}C]mevalonic acid. Moreover, the profile of various chain length isomers of dolichol in the caput is distinctively different from that in the cauda, the former region having a greater abundance of the longer chain lengths.

The present study demonstrated that tunicamycin caused a dramatic reduction in [^3H]mannose incorporation, particularly in the caput. Nevertheless the tissue apparently continued to synthesize and secrete the same glycoproteins as revealed by polyacrylamide gel electrophoresis. An identical result was obtained using [^3H]fucose as precursor. This confirms results obtained previously with lower concentrations of tunicamycin (Brooks, 1981a) and suggests that the majority of epididymal glycoproteins are of the *N*-linked type and consequently are affected by tunicamycin to the same degree.

The results also indicate that tunicamycin reduces glycosylation of proteins in the epididymis without a marked effect on their synthesis or secretion. This was demonstrated by immunoprecipitation of proteins D and E. In the presence of tunicamycin, these proteins were synthesized largely in their non-glycosylated form and their secretion as unglycosylated proteins remained almost unimpaired. Glycosylation is not a prerequisite for secretion of some glycoproteins in other tissues, but sometimes inhibition of glycosylation can also drastically reduce secretion (Olden, Pratt & Yamada, 1978; Gibson *et al.*, 1980).

The corpus epididymidis has a higher dolichol content and is more active in dolichol synthesis than is the cauda (Wenstrom & Hamilton, 1980), yet this is not reflected in the relative activity of these regions towards the incorporation of [^3H]mannose into secretory glycoproteins *in vitro* (Brooks, 1981a; present study). Attempts to alter the relationship between the segments by preincubation of the tissue before the addition of radioactive precursor served only to enhance incorporation in the caput, but not in the corpus or cauda.

Castration substantially reduced the incorporation of [^3H]mannose and [^3H]fucose (see Brooks, 1981a). In contrast to tunicamycin, the resultant profile of secretory glycoproteins seen on a polyacrylamide gel differed from that in control incubations. In the caput it is striking that two prominent bands migrate slightly slower after castration than corresponding bands in normal animals. It remains to be determined whether this results from modified glycosylation of the same proteins or whether the proteins are entirely unrelated.

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