Structural Studies on the Glycoproteins from Bovine Cervical Mucus

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The depolymerization of bovine cervical glycoprotein resulting from cleavage of disulphide bonds, Pronase digestion and both procedures sequentially was assessed by using gel filtration. Cleavage of disulphide bonds followed by Pronase digestion produced more extensive depolymerization than did either treatment alone, and gel filtration of the products resulted in two major peaks of glycosylated material on Sepharose CL-2B and Sepharose 4B. The glycopolypeptides in both peaks had similar sugar and sulphate compositions, but they migrated to different extents on gel electrophoresis. Electrophoretic studies indicated that both glycopolypeptides were derived from the same glycoprotein molecule and not from a mixture of two similar glycoproteins. Pronase digestion of glycoproteins in which the disulphide bonds had been labelled with iodo-[1-¹⁴C]acetamide revealed that most of the cysteine residues were situated in regions susceptible to Pronase. The results show the presence of two types of structural regions in bovine cervical glycoprotein, namely 'naked' peptide or non-glycosylated regions and glycopolypeptide subunit regions in which glycopolypeptides of two different sizes predominate. Comparison of the cervical glycoproteins isolated from mucus secreted during oestrus and pregnancy, by the methods outlined above, did not reveal any structural differences in the glycoproteins to explain the different physical properties of the mucus secreted under these conditions.

The physical properties of bovine cervical mucus change with the hormonal state of the animal; at oestrus the mucus is thin and viscoelastic, but under progesterone dominance the secretion changes to a thick plastoelastic gel (Glover & Scott-Blair, 1953). These changes in the physical properties of cervical mucus are important in reproductive physiology, because the thin mucus secreted during oestrus is favourable for sperm migration and aligns the direction of sperm migration (Tampion & Gibbons, 1962; Gibbons & Mattner, 1971). In contrast, the thick mucus secreted during pregnancy blocks sperm migration and seals the cervix, thus preventing micro-organisms entering the uterine cavity.

The epithelial glycoproteins isolated from cervical mucus of pregnant cows and cows in oestrus have similar chemical compositions (Gibbons, 1959) and physicochemical properties (Gibbons & Glover, 1959). Although they give rise to viscous solutions these glycoproteins do not account for all the physical properties of the intact mucus, and Gibbons (1969) postulated that a protein component of the gel was required to cross-link these glycoprotein molecules. Later work by Gibbons & Sellwood (1973) suggested that this cross-linking protein was an integral part of the glycoprotein molecule but that it was susceptible to degradation by many of the procedures normally used for glycoprotein isolation.

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In the present study the nature of the structures involved in the macromolecular organization of bovine cervical glycoprotein has been investigated and a comparison has been made of these structures in glycoproteins isolated from mucus secreted during oestrus and pregnancy. The results show that two types of structural regions with different compositions and susceptibilities to proteolytic digestion occur in bovine cervical glycoprotein.

Experimental

Materials

Sepharose CL-2B, Sepharose 4B, Blue Dextran 2000, Dextran T250 and Dextran T40 were supplied by Pharmacia (Great Britain) Ltd., London W.5, U.K. Pronase (activity 45000 PUK units/g) (Nomoto & Narahashi, 1959) and dithiothreitol were obtained from Koch-Light Laboratories, Colnbrook, Bucks., U.K. Iodo[1-¹⁴C]acetamide (52.3 mCi/mmol) was purchased from The Radiochemical Centre, Amersham, Bucks., U.K. Liquid scintillator NE-250 was obtained from Nuclear Enterprises, Sighthill, Edinburgh, Scotland, U.K.

Cervical glycoproteins

Bovine mucus secreted during oestrus was centrifuged at 40000g for 1 h and the supernatant discarded. The gel was washed with 3 × 4 vol. of 0.9%

NaCl by gentle inversion at 4°C. Each wash was carried out for 24 h and the gel was recovered after each wash by centrifugation at 40000g for 1 h. Bovine mucus secreted during pregnancy was treated similarly, except that 4 vol. of 0.9% NaCl was added to the mucus at 4°C for 16 h before the initial centrifugation. Bovine cervical glycoproteins were isolated from the washed gels as described by Gibbons (1959).

Analytical methods

The analytical methods used for the determination of total hexose, fucose, sialic acid, amino sugars, amino acids, sulphate and nitrogen were those described previously (Roberts, 1974). Radioactivity was measured with a Packard Tri-Carb or Phillips liquid-scintillation counter by using scintillation fluid prepared as described by Bray (1960) or liquid scintillator NE 250. Gel electrophoresis was carried out in 1.5% polyacrylamide/0.5% agarose as described by Holden *et al.* (1971). Glycoproteins were stained by a periodic acid/Schiff staining procedure (Zacharius *et al.*, 1969).

Gel filtration

Gel filtration was carried out at 4°C on columns (90 cm × 1.5 cm) of Sepharose CL-2B or Sepharose 4B eluted with 0.9% NaCl at a flow rate of 7 ml/h. Fractions (2.4 ml) were collected and their A_{220} and hexose content determined.

Pronase digestion

Pronase digestion was carried out at 37°C for 24 h in 0.5 M-Tris/HCl buffer, pH 8.0, containing 2 mM-CaCl₂ and a drop of toluene, with initial concentrations of 0.05% (w/v) Pronase and 0.5% (w/v) glycoprotein. Further quantities of Pronase were added after 22 h to give a final concentration of 0.07% (w/v) Pronase.

In some experiments the digestion was extended to 72 h with further additions of Pronase equal to the first after 24 and 48 h, thus increasing the final enzyme/substrate ratio to 0.3 (w/w).

Reduction and alkylation of glycoproteins

Glycoproteins, at a concentration of 1.0% (w/v), were reduced with 0.1 M-dithiothreitol in 0.3 M-Tris/HCl buffer, pH 8.0, containing 6 M-guanidinium chloride. The reduction was carried out at 37°C for 2 h under an atmosphere of nitrogen. Iodoacetamide in 0.3 M-Tris/HCl buffer, pH 8.0, containing 6 M-guanidinium chloride was then added to give a final concentration of 0.36 M-iodoacetamide and the solution incubated at 20°C for 2 h. The solution was dialysed against a constant flow of water at 4°C for

24 h and the non-diffusible material recovered by freeze-drying.

Radioactive labelling of disulphide bonds

Glycoproteins (10 mg) were incubated with 0.01 M-iodoacetamide in 0.8 ml of 0.3 M-Tris/HCl buffer, pH 8.0, for 1 h at 20°C and then 0.2 ml of 0.5 M-dithiothreitol in the same buffer was added, the solution flushed with nitrogen and incubated for 2 h at 37°C. The solutions were dialysed against three changes of water (total volume 90 ml) for 3 days and then alkylated with 0.004 M-iodo[1-¹⁴C]acetamide (0.25 mCi/mmol) in 0.1 M-Tris/HCl buffer, pH 8.0 for 1 h at 20°C. Iodo[1-¹⁴C]acetamide that had not reacted was removed by extensive dialysis, and samples of the dialysed solutions were analysed for radioactivity by liquid-scintillation counting and for hexose by using anthrone (Seifter *et al.*, 1950).

Ultracentrifugation in 6 M-guanidinium bromide

Glycoprotein (10 mg) was dissolved in 6 M-guanidinium bromide (5 ml) and centrifuged at 260000g for 42 h at 20°C as described by Gibbons & Sellwood (1973). The contents of the centrifuge tubes were divided into three equal portions of top, middle and lower layers and the glycoprotein in the lower layer was dialysed exhaustively against water and freeze-dried.

Results

Depolymerization of cervical glycoproteins by disulphide-bond cleavage and Pronase digestion

The depolymerization of bovine cervical glycoproteins produced by cleavage of disulphide bonds, Pronase digestion and both treatments sequentially was assessed by using gel filtration on columns of Sepharose CL-2B (Fig. 1) and Sepharose 4B. The untreated glycoprotein from mucus secreted during pregnancy was mainly excluded from the gel matrix of Sepharose CL-2B, but after cleavage of the disulphide bonds with dithiothreitol and alkylation with iodoacetamide some depolymerization occurred, resulting in two peaks on Sepharose CL-2B (Fig. 1b). Similar elution patterns were obtained whether the column was eluted with 0.9% NaCl solution or 6 M-guanidinium chloride. More extensive depolymerization occurred on Pronase digestion of the cervical glycoprotein (Fig. 1c), but this was not as extensive as the depolymerization produced by sequential treatment of reduction and alkylation followed by Pronase digestion (Fig. 1d). All the products of this sequential treatment penetrated the gel matrix of Sepharose CL-2B, resulting in two major peaks of glycosylated material with molecular weights in

Fig. 1. Depolymerization of bovine cervical glycoprotein assessed by gel filtration on Sepharose CL-2B

Cervical glycoprotein isolated from mucus secreted during pregnancy was subjected to gel filtration on columns (90cm $\times$ 1.5cm) of Sepharose CL-2B after various degradative treatments. (a) Cervical glycoprotein (9.4mg); (b) cervical glycoprotein (8.8mg) reduced with 0.1M-dithiothreitol in 0.3M-Tris/HCl buffer, pH8.0, containing 6M-guanidinium chloride for 2h at 37°C, then alkylated with 0.36M-iodoacetamide in 0.3M-Tris/HCl buffer, pH8.0, containing 6M-guanidinium chloride for 2h at 20°C; (c) cervical glycoprotein (12mg) digested with 0.07% Pronase in 0.5M-Tris/HCl buffer, pH8.0, for 24h at 37°C; (d) cervical glycoprotein (10.8mg) subjected to reduction and alkylation as in (b), then dialysed and digested with Pronase as in (c). The columns were eluted with 0.9% NaCl and samples (1.0ml) of the fractions (2.4ml) collected were assayed for total hexose by using anthrone. The elution positions of Dextran T250 and sucrose are shown by the arrows (1 and 2 respectively). The fractions indicated by the horizontal bars were pooled, dialysed exhaustively against water and freeze-dried.

Table 1. Carbohydrate and sulphate composition of glycopolypeptides obtained from bovine cervical glycoprotein after reduction, alkylation and Pronase digestion (Fig. 1d)

	Composition (% by weight of glycoprotein)	
	Fraction DPG 2	Fraction DPG 3
Fucose	5.4	5.3
Galactose	25.0	25.4
Hexosamine	27.4	26.6
Sialic acid	14.0	13.0
Sulphate	2.6	2.3

excess of 250000 as assessed by comparison with dextran markers. Identical results were obtained when the reduction and alkylation preceded the Pronase digestion and when the order of the sequential treatments was reversed. Gel filtration on Sepharose 4B gave a similar result, but owing to the smaller pore size of this gel support the larger of the two peaks of glycosylated material was excluded from the gel. In addition to the two major peaks of glycosylated material there was a small amount of glycosylated material of lower molecular weight which migrated just ahead of the included volume on Sepharose CL-2B and Sepharose 4B. This material was not observed after reduction and alkylation alone, but did occur after Pronase digestion of cervical glycoprotein. Comparison with a control containing Pronase which had been incubated alone for 24h revealed that the glycosylated material of low molecular weight was not an autodegradative product of Pronase. Incubation of cervical glycoprotein with 0.5M-Tris/HCl buffer, pH8.0, at 37°C for 24h without Pronase did not result in the production of any glycosylated material of low molecular weight.

Disulphide-bond cleavage followed by Pronase digestion and gel filtration of glycoproteins isolated from mucus secreted during oestrus and pregnancy resulted in similar elution patterns on Sepharose 4B. The ratio (w/w) of hexose in the two major peaks was 1:2.0 and 1:1.7 in the products from glycoproteins isolated from mucus secreted during oestrus and pregnancy respectively. Analysis of the glycopolypeptides in the two peaks from the glycoprotein isolated from mucus secreted during pregnancy revealed that they had similar sugar compositions (Table 1).

In the experiments described above Pronase digestion was carried out for 24h with a final enzyme/substrate ratio of 0.14 (w/w). When the Pronase digestion was extended to 72h with further additions of Pronase at 24 and 48h to give a final enzyme/substrate ratio of 0.3 (w/w), more extensive depolymerization occurred than with a 24h digestion, all

Fig. 2. Depolymerization of bovine cervical glycoprotein assessed by gel filtration on Sepharose CL-2B: effect of prolonged Pronase digestion

Cervical glycoprotein (15 mg) isolated from mucus secreted during pregnancy was subjected to gel filtration on columns (90 cm $\times$ 1.5 cm) of Sepharose CL-2B after (a) digestion with 0.15% Pronase in 0.5 M-Tris/HCl buffer, pH 8.0, for 72 h at 37°C and (b) digestion with Pronase for 72 h as in (a) followed by reduction with 0.1 M-dithiothreitol in 0.3 M-Tris/HCl buffer, pH 8.0, containing 6 M-guanidinium chloride for 2 h at 37°C followed by alkylation with 0.36 M-iodoacetamide in 0.3 M-Tris/HCl buffer, pH 8.0, containing 6 M-guanidinium chloride for 2 h at 20°C. The columns were eluted with 0.9% NaCl, and portions (1 ml) of the fractions (2.4 ml) collected were assayed for total hexose by using anthrone.

the products being retarded on Sepharose CL-2B (Fig. 2a). However, reduction and alkylation of the 72 h Pronase digest produced more depolymerization than a 72 h Pronase digestion alone, and the resultant gel-filtration pattern (Fig. 2b) was similar to that obtained by sequential treatment of reduction and alkylation followed by a 24 h Pronase digestion (Fig. 1d).

Electrophoresis of glycoprotein-cleavage products

The two main glycopolypeptides obtained by disulphide-bond cleavage followed by Pronase digestion of cervical glycoprotein (labelled DPG 2 and DPG 3 in Fig. 1d) had different migration characteristics on electrophoresis in 1.5% polyacrylamide/0.5% agarose (Fig. 3). These two glycopolypeptides might have been derived from the same glycoprotein molecule or from two different glycoproteins in the

Fig. 3. Gel electrophoresis of cleavage products from bovine cervical glycoprotein

Electrophoresis was carried out in 1.5% polyacrylamide/0.5% agarose gels and the glycopolypeptides were detected with periodic acid/Schiff reagent. (a) Reduced, alkylated and Pronase-digested glycoprotein, fraction DPG 2 (Fig. 1d); (b) reduced, alkylated and Pronase-digested glycoprotein, fraction DPG 3 (Fig. 1d); (c) Pronase-digested glycoprotein, fraction PG 1 (Fig. 1c); (d) Pronase-digested glycoprotein, fraction PG 2 (Fig. 1c); (e) Pronase-digested glycoprotein, fraction PG 1 (Fig. 1c) subjected to reduction and alkylation; (f) Pronase-digested glycoprotein, fraction PG 2 (Fig. 1c) subjected to reduction and alkylation; (g) reduced and alkylated glycoprotein, fraction DG 1 (Fig. 1b) subjected to Pronase digestion; (h) reduced and alkylated glycoprotein, fraction DG 2 (Fig. 1b) subjected to Pronase digestion; (i) cervical glycoprotein, fraction G 1 (Fig. 1a) subjected to reduction, alkylation and Pronase digestion; (j) cervical glycoprotein, fraction G 2 (Fig. 1a) subjected to reduction, alkylation and Pronase digestion.

original preparation. If two different glycoproteins occurred in the original preparation, then they might have different size distributions, and disulphide-bond cleavage or Pronase digestion might lead to enrichment of products from one of these glycoproteins in the high-molecular-weight fractions obtained by gel filtration. To examine these possibilities the high-molecular-weight fractions obtained by gel filtration of the products from Pronase digestion, disulphide-bond cleavage and from the untreated glycoprotein were subjected to further treatments of disulphide-bond cleavage, Pronase digestion or both treatments sequentially, and examined by electrophoresis (Fig. 3). Both fractions G1 and G2 from the original glycoprotein (Fig. 1a) produced electrophoretic bands corresponding to fractions DPG 2 and DPG 3 after disulphide-bond cleavage and Pronase digestion. Electrophoresis of fractions PG 1 and PG 2 obtained by Pronase digestion of cervical glycoprotein (Fig. 1c) resulted in single bands which migrated slightly slower than fraction DPG 2, but after disulphide-bond cleavage both these fractions produced two bands corresponding to DPG 2 and DPG 3 (Fig. 3). Similarly, Pronase digestion of fractions DG 1 and DG 2 obtained by disulphide-bond cleavage of cervical glycoprotein (Fig. 1b) resulted in two bands corresponding to fractions DPG 2 and DPG 3 on electrophoresis (Fig. 3).

Electrophoresis of fraction PG 3 obtained by

Table 2. *Amino acid composition of bovine cervical glycoprotein from mucus secreted during pregnancy, before and after digestion with Pronase*

Compositions are expressed as $\mu\text{mol}/\text{mg}$ of glycoprotein. Abbreviation: n.d., not determined.

Composition	Undigested glycoprotein	Pronase-digested glycoprotein
Asp	0.081	0.025
Thr	0.397	0.388
Ser	0.201	0.170
Glu	0.130	0.061
Pro	0.142	0.122
Gly	0.122	0.066
Ala	0.166	0.135
Cys	n.d.	n.d.
Val	0.125	0.099
Ile	0.025	0.010
Leu	0.066	0.017
Tyr	0.020	Trace
Phe	0.026	Trace
His	0.036	Trace
Lys	0.051	Trace
Arg	0.054	0.033

Pronase digestion of cervical glycoprotein (Fig. 1c) produced mainly material migrating similarly to fraction DPG 3 and small amounts of material migrating similarly to fraction DPG 2.

Radioactive labelling of disulphide bonds

Three samples of cervical glycoprotein secreted during pregnancy (isolated from three separate pools of mucus) and five samples of cervical glycoprotein secreted during oestrus (one was isolated from a pool of mucus and four from individual cows) were subjected to alkylation with unlabelled iodoacetamide followed by reduction and alkylation with iodo[1- ^{14}C]-acetamide. The radioactivities recovered in the glycoproteins secreted during pregnancy corresponded to 10.8, 12.6 and 13.7 mol of *S*-carboxymethylcysteine/ 10^6 g of glycoprotein, whereas those in the glycoproteins secreted during oestrus corresponded to 10.0, 10.8, 12.7, 12.8 and 13.8 mol of *S*-carboxymethylcysteine/ 10^6 g of glycoprotein. Fractionation by ultracentrifugation in 6M-guanidinium bromide (Gibbons & Sellwood, 1973) of a labelled glycoprotein secreted during oestrus did not decrease the labelled *S*-carboxymethylcysteine content of the glycoprotein.

Pronase digestion of labelled glycoproteins

Samples of the glycoproteins labelled with iodo[1- ^{14}C]acetamide were digested with Pronase for 24 h and the radioactivity in the digest was counted before and after dialysis. The recovery of glycoprotein was assessed by hexose determinations. Some 90–93% of

the radioactivity in the three glycoproteins secreted during pregnancy and the five glycoproteins secreted during oestrus was removed from the non-diffusible material after Pronase digestion, but less than 9% of the hexose was lost on dialysis. The radioactivity remaining in the non-diffusible material after Pronase digestion corresponded to values of 1.2–1.6 mol of *S*-carboxymethylcysteine/ 10^6 g of glycoprotein and no significant differences were detected between glycoproteins secreted during oestrus and pregnancy.

Pronase digestion of an unlabelled sample of glycoprotein secreted during pregnancy resulted in extensive removal of some amino acids, but only small losses of threonine, serine and proline occurred (Table 2).

Discussion

The high-molecular-weight glycoprotein isolated from bovine cervical mucus has been the subject of a number of investigations, and although there is general agreement on the composition of this glycoprotein, its detailed structure remains to be elucidated (Gibbons, 1978). The results of the present study are best explained on the basis of two types of structural regions in bovine cervical glycoprotein, namely non-glycosylated or 'naked' peptide regions and glycopolypeptide subunit regions. The 'naked' peptides contain most of the cysteine residues and are susceptible to Pronase digestion, whereas the glycopolypeptide subunits are not cleaved by Pronase. Glycopolypeptide subunits of two different sizes are found in bovine cervical glycoprotein, and in this respect it differs from human bronchial glycoprotein, which has previously been shown to be composed of glycopolypeptide subunits and 'naked' peptide regions (Roberts, 1976, 1978). The existence of two structural regions has previously been postulated in blood-group substances (Dunstone & Morgan, 1965; Kristiansen & Porath, 1968; Donald, 1973), pig gastric glycoprotein (Scawen & Allen, 1977; Allen, 1978) and in bovine cervical glycoprotein (Gibbons & Sellwood, 1973; Masson, 1973; Gelman & Vered, 1976). The pig gastric glycoprotein differs from both human bronchial and bovine cervical glycoprotein in that disulphide-bond cleavage or proteolysis alone is sufficient to cleave the glycoprotein into its individual glycopolypeptide subunits. Scawen & Allen (1977) therefore proposed that the non-glycosylated regions functioned to join four glycopolypeptide subunits via disulphide bridges. Disulphide-bond cleavage or Pronase digestion did not produce equivalent depolymerization of bovine cervical glycoprotein (Fig. 1) and the results indicate that the 'naked' peptide regions are interspersed between glycopolypeptide subunits and join these by peptide linkages. Furthermore, two or more of the long chains thus produced are linked by disulphide bonds, resulting in a structure

similar to that postulated for human bronchial glycoprotein (Roberts, 1978).

The increased depolymerization of bovine cervical glycoprotein observed after combined disulphide-bond cleavage and Pronase digestion compared with Pronase digestion alone (Fig. 1) suggests that disulphide bonds in regions relatively resistant to Pronase are important in cross-linking glycopoly-peptide subunits. However, the labelling studies with iodo[1-¹⁴C]acetamide show that only small amounts of these Pronase-resistant cysteine residues occur in the glycoprotein. Extension of the Pronase digestion to 72 h with increased amounts of Pronase (Fig. 2) resulted in more extensive depolymerization than a 24 h digestion, thus indicating that the polypeptide chains containing these cysteine residues are not completely resistant to Pronase digestion. No detectable differences were observed on gel filtration of products from the combined disulphide-bond cleavage and Pronase digestion of bovine cervical glycoprotein whether the Pronase digestion was carried out for 24 h (Fig. 1d) or 72 h (Fig. 2b). Therefore some of the cysteine residues involved in cross-linking the glycopoly-peptide subunits are probably situated near the junction regions of the 'naked' peptides and glycopoly-peptide subunits. The slow release of these cysteine residues by Pronase digestion could be a result of steric hindrance by the oligosaccharide units.

The two major glycopoly-peptide subunits isolated from cervical glycoprotein have similar sugar and sulphate contents. Comparison of the elution characteristics of the glycopoly-peptide subunits on Sepharose gels with those of dextrans shows that the smaller of the two major glycopoly-peptide subunits is larger than a dextran of mol.wt. 250 000. In addition to the two major glycopoly-peptide subunits a small amount of glycosylated material of low molecular weight was released by Pronase digestion of bovine cervical glycoprotein (Fig. 1c). This was not released by cleavage of disulphide bonds, and the amount released by Pronase digestion did not increase on extending the digestion time to 72 h. Consequently it is considered probable that a small glycopoly-peptide is attached to some of the major glycopoly-peptide subunits via a 'naked' peptide region.

Gel-electrophoretic studies (Fig. 3) on subfractions of the bovine cervical glycoprotein and on the high-molecular-weight fractions obtained from it by cleavage of disulphide bonds or Pronase digestion indicate that the two major glycopoly-peptide subunits are not derived from two different glycoproteins. The number of glycopoly-peptide subunits linked together in the bovine cervical glycoprotein molecule has not been determined and it is uncertain whether mucus glycoproteins are clearly defined molecules with fixed numbers of 'naked' peptides and glycopoly-peptide subunits or whether they are collections of molecules containing varying amounts of these

regions. The latter alternative, superimposed on the microheterogeneity due to variation in the degree of completion of oligosaccharide side-chain biosynthesis, could supply an explanation for the high degree of polydispersity of the mucus glycoproteins (Gibbons, 1972).

Cleavage of the disulphide bonds in a glycoprotein isolated from cervical mucus of the bonnet monkey (*Macaca radiata*) lowered the molecular weight from 1×10^6 to 0.5×10^6 (Hatcher *et al.*, 1977). However, the subunits thus produced were not subjected to proteolysis, so it is not known whether 'naked' peptides are involved in linking together smaller subunits in this glycoprotein. Cervical glycoproteins from different species differ with respect to size of glycopoly-peptide subunits and susceptibility of the cysteine residues involved in interchain disulphide bonding to proteolytic cleavage (G. P. Roberts, unpublished work).

Gel filtration of the products obtained by disulphide-bond cleavage and Pronase digestion from cervical glycoprotein secreted during oestrus did not reveal any significant differences from similar treatments of the glycoprotein secreted during pregnancy. Furthermore, labelling experiments with iodo[1-¹⁴C]-acetamide revealed that the two glycoproteins had similar numbers of disulphide bonds. It is significant that, although the physical properties of the native mucus from pregnant cows and cows in oestrus differ considerably, on soaking the former in 0.9% NaCl overnight, water is taken up to produce a mucus that appears similar to the latter. Consequently the differences in physical properties of the two types of mucus may arise by hormonal control of the state of hydration of the mucus during secretion rather than by structural differences in the component glycoproteins. This possibility is supported by the observation that mucus secreted during pregnancy contains a much higher concentration of glycoprotein than mucus secreted during oestrus.

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