10 Steffens, G.J., Gunzler, W.A., Otting, F., Frankus, E., and Flohe, L., Hoppe-Seylers Z. physiol. Chem. *363* (1982) 1043.
11 Johnson, A.J., Soberano, M., Ong, E.B., Levy M., and Schoellmann, G., in: Thrombosis and Urokinase, p. 59. Ed. R. Paeletti and S. Sherry. Academic Press, New York 1977.
12 Lesuk, A., Terminiello, L., Traver, J.H., and Groff, J.L., Fedn Proc. *26* (1967) 647.
13 Barlow, G.H., Francis, C.W., and Marder, V.J., Thromb. Res. *23* 541 (1981).
14 Sumi, H., Mihara, H., and Toki, N., Thromb. Haemostas. *48* (1982) 342.
15 Sumi, H., Sasaki, K., and Muramatu, M., Acta haemat., jap. *41* (1978) 766.
16 Claeson, G., Fribeger, P., Knos M., and Erikson, E., Haemostas. *7* (1978) 76.
17 Chase, J. Jr, and Shaw, E., Biochemistry *8* (1969) 2212.
18 Wohl, R.C., Summaria, L., and Robbins, K.C., J. biol. Chem. *254* (1979) 9063.
19 Weber, K., and Osborn, M., J. biol. Chem. *244* (1969) 4406.
20 Vesterberg, O., and Svensson, H., Acta chem. scand. *20* (1966) 820.
21 Gros, C., and Labouesse, B., Eur. J. Biochem. *7* (1969) 463.
22 Sumi, H., Toki, N., Maehara, S., Kosugi, T., Akazawa, K., Matsuo, O., and Mihara, H., Acta haemat. *67* (1982) 263.
23 Sumi, H., Takada, Y., and Takada, A., Thromb. Res. *11* (1977) 747.
24 Sumi, H., Toki, N., Takasugi, S., Maehara, S., Maruyama, M., Akazawa, K., Matsuo, O., and Mihara, H., Thromb. Haemostas. *47* (1982) 14.

0014-4754/85/121546-03$1.50 + 0.20/0

Presence of serum proteins in submaxillary duct perfusate

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Summary. The secretory activity of the main excretory duct of rat submaxillary gland was investigated by the technique of luminal perfusion. Immunologic studies of the perfusate revealed the presence of serum antigens and the absence of intrinsic submaxillary gland antigens. It is suggested that the submaxillary duct permits passive transport of serum proteins to saliva from serum.
Key words. Rat submaxillary gland; saliva; serum proteins; submaxillary duct perfusion; serum antigens.

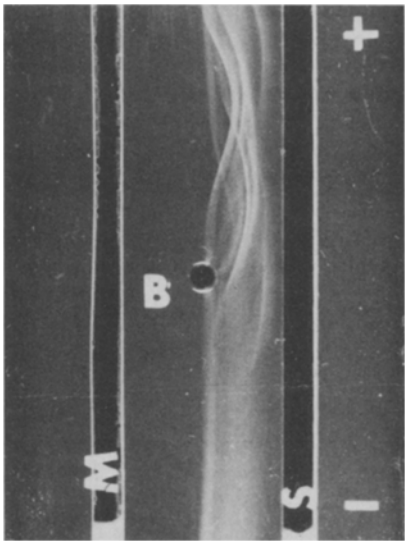
Recent studies have shown evidence of appreciable transport of electrolytes in the main excretory duct of the rat submaxillary gland[1]. Physiological investigations using the technique of luminal perfusion have shown that the rat submaxillary duct actively absorbs sodium and secretes potassium and bicarbonates, and is capable of converting isotonic perfusion fluid to a hypotonic end product[2-4]. In the present study the technique of luminal perfusion was utilized in an effort to evaluate the role of the main submaxillary duct in the secretion of proteins.

Materials and methods. Adult male Long-Evans rats, 4–6 months old, were used in the experiments. The animals were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). For perfusion of the main excretory duct of the submaxillary gland the surgical procedure previously described was followed[5]. The oral end of the right submaxillary duct was first cannulated, using fine polyethylene tubing (Clay-Adams PE 10), to a depth of approximately 3 mm. An opening was made in the skin over the submaxillary-sublingual gland complex, and the hilar end of the submaxillary duct was separated from the sublingual duct. A diagonal cut was made in the submaxillary duct, and a polyethylene cannula with a tip diameter of approximately 100 µl, or less, was inserted in the lumen and ligated in place. The trachea was cannulated to maintain a clear airway.

The submaxillary duct was perfused in situ using a Harvard pump set to deliver fluid from a 0.5-ml glass syringe at a constant rate of 162 nl/min. Normal saline was used for perfusion. Samples of perfusate were collected by attaching short lengths of PE 50 tubing over the PE 10 tubing of the oral cannula and 10-µl micropipettes were used for collecting the samples. Perfusion continued for 60 min for each animal. In some experiments perfusion was carried our after injection of isoproterenol HCl (5–6 mg/rat, i.p.).

Antisera were prepared by injecting saline extract of submaxillary gland s.c. into rabbits as previously described[6]. The extract was incorporated into Freund's adjuvant for the first injection and into incomplete adjuvant for subsequent injections. Antiserum to rat serum was prepared in rabbits in a similar manner. Antiserum to rat submaxillary gland extract was absorbed with lyophilized rat serum in a concentration of 80 mg/ml, which was found by preliminary gel diffusion tests to be a suitable concentration for neutralization. Total protein content of perfusion samples was determined by the method of Lowry et al.[7] using bovine serum albumin for establishing a standard curve. Immunoelectrophoresis was carried out according to the method of Grabar and Burtin[8] as modified by Scheideggar[9] using 1% agarose in 0.05 M barbital buffer at pH 8.6. After the antigens were placed in the wells, a constant current of 4 mA per slide was applied for 2 h. Diffusion of antisera was allowed to proceed for 2–3 days.

Results. Immunoelectrophoretic studies of the submaxillary duct perfusate exhibited several lines of precipitation with antisera to extracts of rat submaxillary gland. Absorption of the antisera with lyophilized rat serum abolished the reaction with



Immunoelectrophoresis in agar gel. Antisera in troughs: anti-submaxillary gland extract absorbed with rat serum (M); antirat serum (S). Well B contains sample of submaxillary duct perfusate. Note absence of intrinsic antigens of submaxillary gland origin and the presence of serum antigens.

the perfusate and demonstrated that these antigenic components originated from the serum and that the duct perfusate did not contain any of the intrinsic submaxillary gland antigens. The presence of serum antigens was confirmed by studying the perfusate with antiserum to rat serum. Immunoelectrophoretic studies demonstrated the presence of both serum albumin and globulins in addition to other serum constituents (fig.). Measurements of total protein of perfusate averaged 1.8 g/100 ml ± 0.4 (± SE obtained from nine animals). Neither total protein concentration nor the immunoelectrophoretic pattern of the perfusate was significantly altered by isoproterenol administration.

Discussion. The presence of serum proteins in saliva is well documented[10], but the mechanism of secretion of these components is not understood. However, since isoproterenol had no effect on the passage of the serum proteins through the duct epithelium, it may be concluded from the present data that there is no β-adrenergic control of this event. In fact, from previous work it appears likely that the passage of these serum proteins is not influenced by stimulation of any autonomic receptors.

In previous studies on the whole parotid gland it has been noted that the ratio of albumin and IgG in saliva was about the same as that in serum[11] and that the secretion of these molecules was independent of flow rate and kind of stimulation[12,13]. The present study shows the specific function of the ductal epithelium of the submaxillary gland in the transport of serum proteins. These data demonstrate the passive transport of serum proteins from serum and shows that the duct plays an important role in the transfer of organic as well as inorganic components. The observed absence of intrinsic submaxillary gland antigens in the perfusate shows that the excretory submaxillary duct of the rat does not participate in the secretion of these antigens and demonstrates the absence of salivary contamination in our samples.

1 Acknowledgments. This work was supported by grant DE 02110 from the National Institute of Dental Research, Bethesda, Maryland 20205, USA. This work was performed in the laboratory of Leon H. Schneyer at his suggestion.

2 Martinez, J. R., Holzgreve, H., and Frick, A., Arch. ges. Physiol. *290* (1966) 124.
3 Young, J. A., Fromter, E., Schogel, E., and Hamann, K. F., Arch. ges. Physiol. *215* (1967) 157.
4 Young, J. A., Martin, C. J., Asz, M., and Weber, F. D., Arch. ges. Physiol. *319* (1970) 185.
5 Schneyer, L. H., Am. J. Physiol. *215* (1968) 664.
6 El-Mofty, S., Experientia *30* (1974) 301.
7 Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. biol. Chem. *193* (1951) 265.
8 Grabar, P., and Burtin, P., Analyse Immuno-Electrophorétique. Masson and Cie, Paris 1960.
9 Scheideggar, J. J., Int. Archs Allergy *7* (1955) 103.
10 Ellison, S. A., in: Handbook of Physiology, vol. 2, p. 531. Ed. C. F. Code. Williams and Wilkins, Baltimore 1967.
11 El-Mofty, S., IADR *52* (1974) 511.
12 El-Mofty, S., IADR *51* (1973) 679.
13 El-Mofty, S., and Schneyer, C. A., Proc. Soc. exp. Biol. Med. *158* (1978) 59.

0014-4754/85/121548-02$1.50 + 0.20/0

Isolation and characterization of a high-molecular-weight glycoprotein from the endometrium of porcine uteri

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Summary. A novel glycoprotein was isolated from the endometrium of porcine uteri. This high-molecular-weight glycoprotein consisted of 25% of protein and 73% of carbohydrate. The carbohydrate composition was quite characteristic in that equimolar N-acetylglucosamine and galactose were major constituents. Its unique nature makes it distinguishable from hitherto-reported glycoproteins.

Key words. Neutral glycoprotein; porcine uteri.

We are interested in the glycoproteins of uterine endometrium[1], since implantation may possibly be mediated by interaction between the cell surface of a fertilized egg and the glycoconjugates of the cells or matrices in the endometrium. During the course of studies on glycoproteins from porcine uteri, we have found a hitherto undescribed glycoprotein with a high molecular weight. This paper reports its isolation and partial characterization.

Materials and methods. Porcine uteri were obtained from a local slaughter house. The uteri, freed from adhering tissues, were opened by longitudinal cutting and rinsed with ice-cold 0.9% NaCl. All operations were carried out at 4 °C unless otherwise stated. The endometrium was scraped off from the uteri, weighed and homogenized in 100 volumes of 5 mM EDTA (the pH of the solution was adjusted to 7.0 with 1 N NaOH) with a Waring blender. The homogenate was centrifuged at 20,000 rpm for 2 h. Concentrated potassium phosphate buffer was added to the supernatant (pH 7.0) to make the solution 50 mM. The solution was applied to a column (4 × 22 cm) of DEAE-Sephadex A-25 pre-equilibrated with 50 mM potassium phosphate (pH 7.0), and the column was washed with the same buffer. The eluate and the washing buffer were combined and applied to a column (2.5 × 30 cm) of CM-Sephadex C-25 pre-equilibrated with the same buffer. The column was washed with the same buffer. The eluates and the washings were combined, concentrated to a small volume by ultrafiltration and applied to a column of Sepharose CL-4B pre-equilibrated with the same buffer. The fractions eluted at the void volume were combined, dialyzed and lyophilized.

Electrophoresis on cellulose acetate membranes[2], sodium dodecylsulfate-1% agarose gels[3] and sodium dodecylsulfate-3.3% polyacrylamide gels[3] were carried out as described previously. Hexoses[4], hexosamines[5], sialic acid[5] and amino acids[5] were quantitated according to the methods described previously.

Amino acid composition of the glycoprotein. The data are expressed as residues per 1000 residues

Aspartic acid	69	Methionine	3
Threonine	55	Isoleucine	32
Serine	109	Leucine	62
Glutamic acid	128	Tyrosine	24
Proline	61	Phenylalanine	30
Glycine	158	Lysine	48
Alanine	58	Histidine	20
Valine	77	Arginine	51
Half cystine	5	Tryptophan	10