Carbohydrates of Human Vaginal Tissue

Much has been published on the histology of the human vagina and its changes during the menstrual cycle; however, little is known about its biochemistry and its metabolism. While the presence of glycogen and lactic acid in the human vagina has been known since the latter part of the nineteenth century, no attempt has been made to elucidate the method of synthesis or breakdown of the glycogen molecule.

In a recent publication[1] glucose, maltose, maltotriose, and maltotetraose were identified in human vaginal secretions. The secretions were obtained by placing a cap over the cervical os and inserting a pre-weighed cellulose tampon which remained in the vagina for 24 h. This method of collection did not exclude the possibility of the compounds originating from the bacterial flora normally present in the human vagina.

Normal vaginal tissue was obtained from patients undergoing gynæcological surgery. The tissue was immediately frozen and stored until a total of 35 g was obtained. A water extract was made by homogenizing the tissue in a blender and the carbohydrates extracted by Fishman's method[2]. Aliquots of the extract were chromatographed in a one-dimensional, descending system of either, *n*-butanol/pyridine/water (60 : 40 : 30) or *n*-butanol/acetic acid/water (40: 10 : 50)[3]. The papers were dipped in the modified silver nitrate reagent of Trevelyan[4].

Four discrete spots were observed in the chromatograms (Fig. 1). These were identified as glucose, maltose, maltotriose, and maltotetraose. Confirmation of the identity of the compounds was made by: (1) comparison of R_G and R_F values of the unknowns to standard compounds; (2) acid hydrolysis which yielded only glucose; (3) identification of the 1–4 linkage[5]; (4) plotting the value $1g_{10}$ $(1/R_F-1)$ against hexose units per molecule demonstrating the compounds as members of a homologous series[6].

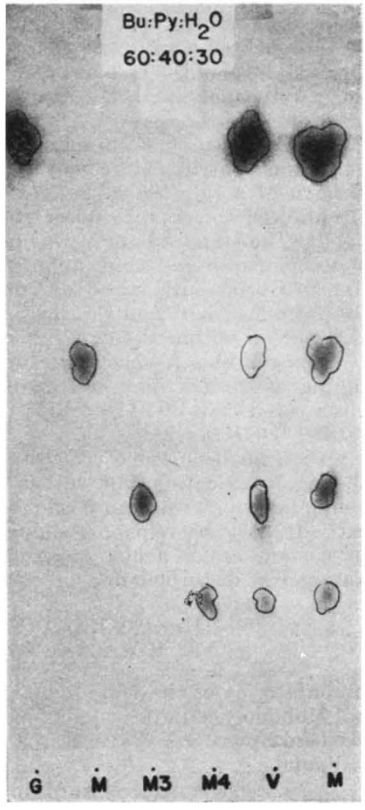

Fig. 1. Separation of the carbohydrates of human vaginal tissue on a one-dimensional descending system of *n*-butanol–pyridine–water, Whatman No. 1 paper. *G*, Glucose; *M*, maltose; M_3, maltotriose; M_4, maltotetraose; *V*, vaginal extract; *M*, mixture of the standard solutions

The oligosaccharides, maltotriose and maltotetraose, have been identified in rat liver[2], rat diaphragm[7], rat muscle extract[8], in the crabs *Cancer magister* and *Hemigrapsus nudus*[9], and in the crayfish *Oreonectes*[10]. The identification of these compounds in vaginal epithelium, a relatively accessible human tissue, which responds to hormonal stimulation, will permit further examination of the oligosaccharides and their role in human carbohydrate metabolism.

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A. T. Gregoire

Strickler Root Laboratory,
Department of Obstetrics and Gynecology,
Jefferson Medical College,
Philadelphia.

[1] Sumawong, V., Gregoire, A. T., Johnson, W. D., and Rakoff, A. E., *Fertil. and Steril.*, **13**, 270 (1962).
[2] Fishman, W. H., and Sie, H. G., *J. Amer. Chem. Soc.*, **80**, 121 (1958).
[3] Giri, K. U., and Nigam, V. N., *J. Ind. Inst. Sci.*, A, **36**, 49 (1954).
[4] Trevelyan, W. E., Proctor, D. P., and Harrison, J. S., *Nature*, **166**, 444 (1950).
[5] Saroja, K., Venkataraman, R., and Giri, K. V., *Biochem. J.*, **60**, 399 (1955).
[6] French, D., and Wild, G. M., *J. Amer. Chem. Soc.*, **75**, 2612 (1953).
[7] Beloff-Chain, A., Catanzaro, R., Chain, E. B., Masi, I., Pocchiari, F., and Rossi, C., *Proc. Roy. Soc.*, B, **143**, 481 (1955).
[8] Olavarria, J., *J. Biol. Chem.*, **235**, 3058 (1960).
[9] Meenakshi, V. R., and Scheer, B. T., *Comp. Biochem. Physiol.*, **3**, 30 (1961).
[10] McWhinnie, M. A., and Saller, P. N., *Comp. Biochem. Physiol.*, **1**, 110 (1960).

Differentiation of Myoglobins and Hæmoglobins

The simultaneous estimation by de Duve[1] of hæmoglobin (Hb) and myoglobin (Mb) in solution, based on the spectral characteristics of their carbon monoxide compounds, requires the solutions to be crystal clear and strongly buffered and the relevant wave-lengths must be reproduced to within a fraction of a mμ.

Brown *et al.*[2] recorded a great difference between the relative stabilities of human hæmoglobin and various myoglobins as estimated by the time required for half conversion to denatured hæmochrome in 0·06 M sodium hydroxide (t_{50}%) and by extrapolation to zero time of the plot of log per cent undenatured pigment against time.

Jonxis and Visser[3] used the ratio of optical densities at 576 mμ before and during alkaline denaturation in determining fœtal hæmoglobin in blood, and a similar method has been found effective for the differentiation of myoglobin and hæmoglobin in solution.

Table 1. Spectrophotometric Properties of Pig Oxymyoglobin and Oxyhæmoglobin during Alkaline Denaturation

Sample	(Light path of cells, 10 mm) Initial optical density at 577 mμ	t_{50}% (min)	$\frac{O.D._1}{O.D._2}$
	Oxymyoglobin		
A	0·58	26	1·49
	0·64	130	1·48
B	0·43	11	1·35
	0·64	27	1·46
C	0·30	69	1·63
	0·45	66	1·64
	0·90	—	1·59
	Oxyhæmoglobin		
A	0·54	36	2·68
B	0·59	47	2·49
	0·59	30	2·39
	0·64	31	2·28
	1·22	29	2·44
C	0·58	42	2·51
	0·72	38	2·52
	1·02	38	2·49
D	0·29	—	2·47
	0·42	—	2·35
	0·88	—	2·42