

to 64 minutes (*P. regina*) of internal carriage. Cysts were found culturable from fecal droplets for periods of from 137 minutes (*P. regina*) to 218 minutes (*C. macellaria*).

*Conclusions.* From the evidence cited above, it seems highly probable that the filth flies studied play no important rôle as transmitters of trophozoites or cysts of *E. histolytica* by external carriage. However, the viability of cysts for periods as long as 3½ hours after initial ingestion and later dejection indicates a potential natural method of transmission.

### 13459

#### Carbohydrates of the Gonadotropic Hormones.

SAMUEL GURIN. (Introduced by D. Wright Wilson.)

*From the Department of Physiological Chemistry, School of Medicine, University of Pennsylvania, Philadelphia.*

The presence of carbohydrate in various purified gonadotropic hormone preparations raises a number of questions concerning the significance of the carbohydrate and its relationship to biological activity. Evans, *et al.*, have studied the carbohydrate of various pituitary<sup>1, 2</sup> as well as pregnant mare serum preparations.<sup>3</sup> McShan and Meyer<sup>4</sup> have recently called attention to the possible significance of carbohydrate in their studies of pituitary follicle stimulating hormone preparations. The nature of the carbohydrate in the gonadotropic hormone of urine of pregnancy has likewise been studied.<sup>5</sup>

The data reported by Evans, *et al.*,<sup>2</sup> indicate that the interstitial cell stimulating hormone of the pituitary contains mannose, while a preparation made from pregnant mares' serum was found to contain galactose. Studies along these lines have been carried out in this laboratory and a comparison of the carbohydrate in various gonadotropic hormones is reported here.

The carbazole method<sup>6</sup> was employed for the identification and

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<sup>1</sup> Evans, H. M., Fraenkel-Conrat, H., Simpson, M. E., and Li, C. H., *Science*, 1939, **89**, 249.

<sup>2</sup> Li, C. H., Simpson, M. E., and Evans, H. M., *Endocrinology*, 1940, **27**, 803.

<sup>3</sup> Li, C. H., Evans, H. M., and Wonder, D. H., *J. Gen. Physiol.*, 1940, **23**, 733.

<sup>4</sup> McShan, W. H., and Meyer, R. K., *J. Biol. Chem.*, 1940, **135**, 473.

<sup>5</sup> Gurin, S., Bachman, C., and Wilson, D. W., *J. Biol. Chem.*, 1940, **133**, 467.

<sup>6</sup> Gurin, S., and Hood, D. B., *J. Biol. Chem.*, 1939, **131**, 211.

TABLE I.

Hormone	Concentration mg/cc	€520/€420		Hexose present %	Hexosamine %	Hexose	
		€540	€520/€420			Hexosamine	
Pituitary L. H.	1.901	.159	0.66	Mannose	2.8	2.2	1.27
" F. S. H.	0.998	.130	0.73§	"	4.5	4.4	1.02
Equine Gonadotropin*	1.270	.627	1.00	Galactose	15.6	8.3	1.88
" " †	1.430	.755	1.10	"	17.6	8.4	2.10
Human Pregnancy Urine Gonadotropin			0.95-1.05	"	10-12	5-6	2
Non-Pregnant Mare Serum‡	1.100	.307	0.80	Mannose- Galactose	10.0		

\*Preparation No. 18-5-1; assay 3300 R.U./mg. For a discussion of the biological assay method see reference 11.

†Preparation No. 15-111-1; assayed 4000 R.U./mg.

‡Kindly supplied by Dr. H. Goss.

§This value is slightly higher than the one usually obtained with pure mannose. It suggests the presence of a small amount of another hexose.

estimation of the sugars. The Palmer-Smyth-Meyer<sup>7</sup> modification of the Elson-Morgan procedure<sup>8</sup> was employed in the estimation of hexosamine after preliminary hydrolysis in sealed tubes with 4 N HCl for 8 hours at 95-100°. The results are summarized in Table I.

The luteinizing and follicle-stimulating hormones prepared from pituitary glands were generously supplied by Dr. H. B. van Dyke. They represent highly purified fractions prepared by the method of Greep, van Dyke and Chow.<sup>9</sup> It will be observed that the ratios €520/€420 indicate that mannose is the sole or main hexose present in these preparations. The molecular ratios of hexose to hexosamine are in both instances close to unity indicating that, in these preparations, the carbohydrate is composed of mannose and hexosamine in equimolar proportions. These results are in harmony with the findings reported by Evans<sup>2</sup> for the carbohydrate of the interstitial cell stimulating hormone. The total carbohydrate in these fractions is somewhat lower, however, than the values obtained by Evans.

Two samples of gonadotropic hormone obtained from pregnant mare serum were kindly furnished by Dr. H. Goss. These preparations were made by the method described by Goss and Cole.<sup>10</sup> The €520/€420 ratios obtained with these samples indicate that the hexose present is either galactose or an equimolar mixture of glucose and mannose. The carbohydrate in these preparations appears to contain hexose and hexosamine in a molar ratio of 2:1.

<sup>7</sup> Palmer, J. W., Smyth, E. M., and Meyer, K., *J. Biol. Chem.*, 1937, **119**, 491.

<sup>8</sup> Elson, L. A., and Morgan, W. T. J., *Biochem. J.*, 1933, **27**, 1824.

<sup>9</sup> Greep, R. O., van Dyke, H. B., and Chow, B. F., *J. Biol. Chem.*, 1940, **133**, 289.

<sup>10</sup> Goss, H., and Cole, H. H., *Endocrinology*, 1940, **26**, 244.

<sup>11</sup> Cole, H. H., and Erway, J., *Endocrinology*, 1941, **29**, 514.

It is probable that the hexose is actually galactose in view of the findings of Evans<sup>2, 3</sup> although there is some discrepancy concerning the ratio of hexose to hexosamine. This may probably be ascribed to the greater purity of our preparations.

A preparation made from non-pregnant mare serum by a similar procedure proved to contain equimolar amounts of mannose and galactose. In this case the carbohydrate moiety appears to be similar to that present in serum mucoid and in other serum glycoproteins.

It is interesting to note that the carbohydrate groups of the gonadotropins of pregnant mare serum and of urine of pregnancy in women are apparently similar and that they are different from those found in the pituitary gonadotropic hormones. It is difficult to believe that these results are purely fortuitous and that the presence of a different type of carbohydrate in the gonadotropins associated with pregnancy (gestation) is not a result of the marked metabolic changes occurring during pregnancy.

*Summary.* A qualitative as well as a quantitative study has been made of the hexose present in the gonadotropic hormones of the pituitary gland, pregnant mare serum and human pregnancy urine. The hormones obtained from the pituitary gland contain mannose and hexosamine in equimolar proportions. The gonadotropins of pregnant mare serum and human pregnancy urine appear to contain galactose rather than mannose. In these preparations the molar ratio of hexose to hexosamine is 2:1.

### 13460 P

#### **Influence of Estrogen on Respiration of Rat Uterine Tissue.**

WILLIAM R. CARROLL. (Introduced by F. L. Hisaw.)

*From the Biological Laboratories, Harvard University, Cambridge, Massachusetts.*

Although much is known about the morphological changes that take place in various tissues and organs due to the influence of the different sex hormones, little is known about true physiological effects, and still less about the actual biochemical action of the active substances. Hence an investigation has been undertaken to locate the mode and place of action in a physiological system of one of the female sex hormones, estradiol.

One of the physiological effects of estrogen, and one that lends itself to biochemical analysis, is the increase in oxygen consumption