

contained only follicles. The uteri of these rats were distended with fluid in most cases while the vaginae of approximately two-thirds of the rats were not open. The supercentrifuged aqueous extract before digestion with trypsin, however, produced ovaries which contained many corpora lutea together with undistended uteri and open vaginae in all rats. These follicle-stimulating preparations were found to be free also of lactogenic and thyrotropic activities as indicated by the pigeon and young chick tests respectively.

Summary. A convenient method for the preparation of follicle-stimulating extracts from sheep pituitary powder by use of trypsin is described briefly. These preparations produced follicles only, when tested on normal female rats for 4.5 days, and were free of lactogenic and thyrotropic activities.

10543 P

Carbohydrate Properties of Pituitary Follicle-Stimulating and Luteinizing Preparations.*

W. H. MCSHAN AND ROLAND K. MEYER.

From the Department of Zoology, University of Wisconsin.

We reported in a previous publication that the follicle-stimulating activity of sheep pituitary extract is destroyed by certain amyolytic enzymes, and on the basis of this evidence we suggested that the follicle-stimulating activity might be due to, or dependent upon, a carbohydrate grouping.¹ Such a point of view logically led to an examination of the carbohydrate properties of our follicle-stimulating and luteinizing hormone preparations. Anticipation of a part of our findings by Evans, *et al.*,² prompts us to publish a brief report of certain of our results at this time.

Follicle-stimulating preparations made by the trypsin method³ and luteinizing extracts which were strong in thyrotropic activity were hydrolyzed with hydrochloric acid and the total reducing action of

* Supported in part by a grant from the Wisconsin Alumni Research Foundation.

¹ McShan, W. H., and Meyer, R. K., *J. Biol. Chem.*, 1938, **126**, 361.

² Evans, H. M., Frankel-Conrat, H., Simpson, M. E., and Li, C. H., *Science*, 1939, **89**, 249.

³ McShan, W. H., and Meyer, R. K., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **40**, 699.

the hydrolysates determined. In order to show the striking difference between the follicle-stimulating and luteinizing preparations the reducing action of the hydrolysates is expressed in terms of glucose which, however, does not infer that the carbohydrate involved in these extracts is glucose. The hydrolysates of the follicle-stimulating preparations gave reducing action equivalent to 1.22 mg of glucose per 0.5 g equivalent of pituitary powder as compared to 0.38 mg equivalent of glucose for the hydrolysates of the luteinizing extracts. The total reducing action expressed as glucose per 0.5 g equivalent of the dialyzed follicle-stimulating extracts was 20.3% of the total protein content of these extracts, which was 6 mg of protein per 0.5 g equivalent of pituitary powder based on Kjeldahl nitrogen determinations.³ The value of 20.3% for our follicle-stimulating extracts was decreased, however, to 18.7% when the hydrolysates were treated with zinc hydroxide before the reducing action was determined.⁴ This decrease with the zinc hydroxide treatment suggests that the value of 20.3% for the total reducing action was due in part to other reducing substances than carbohydrate. This leads to the belief that the value of 18.7% expressed as glucose is more nearly correct for our follicle-stimulating extracts. Furthermore, the basis on which the carbohydrate values were determined and calculated may account for the remaining difference between our value of 18.7% and that of 13% given by Evans, *et al.*,² for their follicle-stimulating extract.

The follicle-stimulating preparations used in the above reduction experiments gave a strong Molisch test for carbohydrate while the luteinizing extracts gave a weak test, which is in agreement with the difference in the reducing values for these respective preparations. The carbohydrate contained in the follicle-stimulating preparations became dialyzable on completion of the hydrolysis.

Another experiment was done in which the follicle-stimulating preparations were inactivated by electro dialysis while the luteinizing extracts were not inactivated. During the treatment of the follicle-stimulating extracts a precipitate formed which contained a small amount of carbohydrate while the most of the carbohydrate remained in the soluble fraction. The inactivation of these follicle-stimulating extracts on electro dialysis, may involve, therefore, a partial disruption of the carbohydrate grouping.

Further study was made of the inactivation of the follicle-stimulating activity by digestion of the extract with dialyzed ptyalin.¹ The digest was dialyzed after which the dialysate was concentrated. The total reducing action was determined on the concentrated dialysate and

⁴ Somogyi, M., *J. Biol. Chem.*, 1930, **86**, 655.

calculated in terms of glucose, which was equivalent to 0.056 mg per 0.5 g equivalent of the follicle-stimulating extract. The concentrated dialysate gave a positive Molisch test.

The results from the enzyme experiment described in which a part of the carbohydrate of the follicle-stimulating extracts became dialyzable on inactivation with ptyalin and the experiment on electro-dialysis in which there was inactivation with a partial separation of the carbohydrate, do not prove, but further substantiate our previous suggestion that the follicle-stimulating activity may be associated with, or dependent upon a carbohydrate grouping.

Summary. The carbohydrate content of our follicle-stimulating and luteinizing preparations is given in terms of glucose calculated from the reducing action of the hydrolysates. Results from enzymatic inactivation and electro-dialysis are given which suggest that the follicle-stimulating activity may be associated with a carbohydrate grouping.

10544

Protective Action of Sulfapyridine in Rabbits Infected with Pneumococci.*

W. P. LARSON, RAYMOND N. BIETER AND MILTON LEVINE.

From the Departments of Bacteriology and Pharmacology, University of Minnesota.

Most of the studies on the protective action of sulfanilamide and sulfapyridine on streptococcic and pneumococcic infections have been made on white mice. In the work here reported rabbits were used as the experimental animals. Intracutaneous inoculations were made in order to permit the observation of differences in the local lesions occurring in the treated and the control groups.

Rabbits weighing approximately 2 kilos were given 0.3 cc of a 1-100 dilution of an 8-hour culture of a Type II pneumococcus. The strain used had been transferred alternately through mice and veal broth for more than a year.

Intraperitoneal injections of 0.2 cc into mice, in dilutions of 1:10,000,000, kills 50% of the animals.

Sulfapyridine, 2-(sulfanilamido)-pyridine, was administered orally by suspending in 10% acacia and permitting the suspension to

* Aided by a grant from the research funds of the Graduate School. Merck & Co. kindly supplied the sulfapyridine used in these experiments.