Wiley and Sons, Inc., New York, 1960, p320.
3. Eriksson, S., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 582.
4. Thompson, J. C., Vars, H. M., *ibid.*, 1953, v83, 246.
5. Van Zyl, A., *J. Endocrinol.*, 1957, v16, 213.
6. Sjövall, J., *Clin. Chim. Acta*, 1959, v4, 652.
7. Bergström, S., Danielsson, H., *Acta physiol. Scand.*, 1958, v43, 1.

Received December 21, 1961. P.S.E.B.M., 1962, v109.

Prolactin, Immunologic Evidence of Species Specificity.* (27302)

RICHARD P. LEVY AND JAMES SAMPLINER (Introduced by I. Rothchild)
Department of Medicine, Western Reserve University School of Medicine and University Hospitals, Cleveland, Ohio

Previous studies from this laboratory demonstrated the antigenicity of ovine prolactin in rabbits and indicated that the antiserum produced seemed to be relatively specific for prolactin when immunization was done with a purified prolactin preparation(1). The present study was designed to test the antigenicity of a relatively crude preparation of human prolactin and to determine its immunologic cross-reaction with ovine prolactin. Such information would assess the feasibility of an immunoassay for human prolactin.

Materials and methods. Ovine Prolactin: A purified preparation of lactogenic hormone with a stated potency of 15 I.U./mg was used.†

Human Prolactin: A preparation from human anterior pituitary glands with a stated potency of 10 I.U./mg was used.‡

Immunization: Two adult white rabbits were injected subcutaneously with saline solutions of ovine and human prolactin, respectively, suspended in equal volumes of Freund's complete adjuvant. Each dose contained approximately 50 I.U. of prolactin activity. Seven weeks later intravenous booster injections of 0.20 mg of the particular prolactin in saline solution were given.

Sampling: Peripheral blood samples were obtained from the rabbits just prior to immunization and one week after the booster injections.

Hemagglutination titers: The technic employed was that described previously(1), except that each 1.0 ml volume of a 2.5% suspension of washed, tannic acid treated sheep erythrocytes was sensitized with only 0.10 mg of prolactin.

Absorption of antiserum: 1.0 ml of human growth hormone§ was added to 4.0 ml of the antiserum daily. The mixture was refrigerated for 24 hours, centrifuged, and the precipitate removed. This procedure was repeated until no further precipitate was visible.

Results. Both animals lacked demonstrable anti-prolactin antibodies before immunization; both developed such antibodies after immunization. Table I summarizes the results of hemagglutination studies with the antisera. When reacted with the homologous antigen, significant titers occurred; hemagglutination was absent when the sensitized

TABLE I. Prolactin, Anti-prolactin Hemagglutination.

Type of anti-prolactin antiserum	Source of prolactin, erythrocyte sensitization	Hemagglutination titers*
Ovine	Ovine	1/640
"	Human	0
Human	"	>1/10,000†
"	Ovine	0

* The same titers were observed in duplicate experiments done on different days.
† Higher dilutions of antiserum were not tested.

* Supported in part by grant from Nat. Inst. Health, P.H.S.

† NIH prolactin, Lot U 10303, supplied through the kindness of the Endocrine Study Section, Nat. Inst. Health, U.S.P.H.S.

‡ Prepared and bioassayed by Dr. W. H. McShan, Univ. of Wisconsin, from acetone dried human anterior pituitaries.

§ Purified human growth hormone (Lot Homo No. 9), prepared by Dr. M. Raben(2), and diluted to a concentration of 10 μg/ml.

erythrocytes were reacted with the heterologous antigen.

The specificity of the hemagglutination caused by the reaction of anti-ovine prolactin antiserum with ovine prolactin-sensitized erythrocytes was evaluated by determining the amount of test tubstances added to the antiserum that inhibited hemagglutination. Neither 1.0 mg of the human prolactin preparation nor 5.0 mg of mammotrophic tumor extract‖ inhibited the reaction, while as little as 0.20 milliunit of ovine prolactin measured simultaneously, significantly inhibited the antigen-antibody reaction.

To further substantiate this apparent species specificity, diffusion studies with Ouchterlony plates and cellulose acetate discs(3) were performed. Anti-ovine prolactin antiserum reacted with ovine prolactin to cause a relatively broad single band of precipitation, while the human prolactin and the antiserum produced with it reacted to form 2 lines, a faint one close to the center well, containing antiserum, and a denser broader band closer to the antigen well. When the anti-human prolactin antiserum absorbed with human growth hormone was tested against human prolactin only the denser outer band was visible. No cross-reaction between the materials of ovine and human origin was seen. The cellulose acetate discs gave similar results; both technics failed to show reaction between prolactin from either species and antiserum produced by immunization with the prolactin preparation derived from the heterologous species.

Discussion. Failure to obtain immunologic lactin from either species and antiserum prolactin suggests that a highly purified human preparation with its homologous antiserum is a prerequisite for development of an immunoassay for human prolactin. The assay system with ovine prolactin detected as little as 0.013 μg of the prolactin preparation. Based on the bioassay system of Simkin and Goodart(4), if a parallel system could be constructed with human prolactin, its sensitivity with specific antisera would be sufficient to evaluate prolactin levels in human blood.

‖ Kindly provided for testing by Dr. J. Furth, Roswell Park Memorial Inst., Buffalo, N. Y.

Li has cited differences in solubility behavior and tyrosine content between ovine and beef prolactin(5). Recently Ferguson and Wallace(6) have also shown electrophoretic differences between these 2 prolactins. The inability to detect prolactin-like activity in extracts from rat mammotrophic tumors adds further weight to the probability that the prolactins of different species have structural differences.

Diffusion studies in semi-solid media further demonstrated the immunologic species specificity of human and ovine prolactin. Absorption of anti-human prolactin antiserum with human growth hormone caused disappearance of one precipitin line. The human prolactin preparation thus also contained antigens similar to some of those in human growth hormone. While the present study clearly indicates an immunologic difference between the prolactins of different species, it does not permit any definite conclusions regarding the degree of identity between growth hormone and prolactin. This latter point would be clarified by evaluating the ability of an antiserum against one of the hormones to neutralize the biologic activity of the other.

Summary. Rabbits immunized either with preparations of human or ovine prolactin developed antibodies against these hormones. As measured by the technic of hemagglutination and by diffusion in semi-solid media the antibodies against the ovine preparation did not react with the human material, nor did the antiserum against the human material react with the ovine prolactin preparation. These findings add to the evidence suggesting the species specificity of prolactin. It appears that a satisfactory immunoassay for human prolactin will require a highly purified preparation of the human material as the antigen.

1. Levy, R. P., Sampliner, J., PROC. SOC. EXP. BIOL. AND MED., 1961, v106, 214.

2. Raben, M., *Science,* 1957, v125, 883.

3. Consden, R., Kohn, J., *Nature,* 1959, v183, 1512.

4. Simkin, B., Goodart, D., *J. Clin. Endocrinol. and Metab.,* 1960, v20, 1095.

5. Li, C. H., *Postgrad. Med.,* 1961, v29, 13.

6. Ferguson, K. A., Wallace, A. L., *Nature* (London) 1961, v190, 629.

Received December 21, 1961. P.S.E.B.M., 1962, v109.