tomized mice with allogenic thymus grafts in cell-tight millipore chambers(23) offer evidence that a humoral factor may mediate the influence of the thymus on the peripheral lymphoid system, and evidence for such a humoral factor has recently been adduced in adult animals(24). From this point of view, the state of the peripheral lymphatic tissue may exert a controlling influence on thymic cell production. Experiments are currently in progress to evaluate the relative importance of the several mechanisms presented above in the regeneration of the thymus and the state of the remainder of the lymphoid system.

Summary. Administration of specific rabbit anti-rat lymphocyte gamma globulin (IgG) produced prolonged severe lymphopenia in rats made tolerant to normal rabbit IgG. A decrease in thymic weight due to loss of small lymphocytes occurred. Combined morphologic and biochemical studies demonstrated marked proliferative activity of the precursor cells. The anti-lymphocyte IgG did not appear to gain entrance into the thymus.

1. Andreasen, E., in The Kinetics of Cellular Proliferation, Stahlman, F., Jr., ed., Grune & Stratton, New York, 1959, p19.

2. Metcalf, D., in The Thymus in Immunobiology, Good, R. A., & Barielson, A. E., ed., Hoeber-Harper, New York, 1964, p150.

3. Sainte-Marie, G., Leblond, C. P., Proc. Soc. Exp. Biol. & Med., 1958, v97, 263.

4. Frenkel, E. P., Sugino, Y., Bishop, R. C., Potter, R. L., Radiation Res., 1963, v19, 701.

5. Sainte-Marie, G., Leblond, C. P., Blood, 1964, v23, 275.

6. Craddock, C. B., Nakai, G. S., Fukuta, H., Vanslager, L. M., J. Exp. Med., 1964, v120, 389.

7. Cross, A. M., Leuchars, E., Miller, J. F. A. P., J. Exp. Med., 1964, v119, 837.

8. Hildemann, W. H., Linscott, W. D., Morlina, M. J., in Ciba Foundation Symposium on Transplantation, Churchill, C., London, 1962, p236.

9. LoSpalluto, J., Miller, W., Dorward, B., Fink, C. W., J. Clin. Invest., 1962, v41, 1415.

10. Denman, A. M., Frenkel, E. P., Ziff, M., Fed. Proc., 1965, v24, 305.

11. Sugino, Y., Frenkel, E. P., Potter, R. L., Radiation Res., 1963, v19, 682.

12. Nossal, G. J. V., Ann. N. Y. Acad. Sci., 1964, v120, 171.

13. Ernström, U., Gyllensten, L., Larsson, B., Nature, 1965, v207, 540.

14. Potter, R. L., Schlessinger, S., Buettner-Janush, V., Thompson, L., J. Biol. Chem., 1957, v226, 381.

15. Hamilton, L. D., Nature, 1956, v178, 597.

16. Trowell, O. A., J. Biophys. Biochem. Cytol., 1957, v3, 317.

17. Hulse, E. V., Brit. J. Haem., 1963, v9, 376.

18. Grasbeck, R., Nordman, C., de la Chapelle, A., Lancet, 1963, vii, 385.

19. ———, Acta Med. Scand. Suppl., 1964, v412, 39.

20. Thorbecke, G. J., Keuning, F. J., J. Immunol., 1953, v70, 129.

21. McFarlane, A. S., Nature, 1958, v182, 53.

22. Merritt, K., Johnson, A. G., J. Immunol., 1965, v94, 416.

23. Osoba, D., Miller, J. R. A. P., Nature, 1963, v199, 653.

24. Klein, J. J., Goldstein, A. L., White, A., Proc. Nat. Acad. Sci., U. S., 1965, v53, 812.

Received April 10, 1967. P.S.E.B.M., 1967, v125.

Radioimmunoassay for Human Luteinizing Hormone.* (32295)

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During the past year radioimmunoassays for human luteinizing hormone (LH) have been developed in several laboratories(1-4). In all of these, the basis of the assay rests upon the development of antisera to human chorionic gonadotropin (HCG) and the cross-reactivity of LH with these HCG antisera(5). The labeled antigen used has varied from highly purified pituitary LH(1) to purified

* This study was supported in part by USPHS Grant AM-3758.

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HCG(2-4). The LH standards for these assays generally have been of urinary origin although direct comparison with pituitary standards has been made(1). This communication describes a sensitive double-antibody radioimmunoassay technique for LH using human pituitary extracts prepared in this laboratory for the production of antibodies, for iodination, and for standards.

Materials and methods. Antisera to potent human pituitary LH extracts (1.8-2.8 NIH-LH-S1 units per mg by bio-assay(6)) were prepared in guinea pigs. The animals were bled 14 days after 3 weekly subcutaneous injections of 3 units emulsified in complete Freund's adjuvant and an additional injection in saline.

A highly purified human LH preparation (Ryan-41965B1BA2) was used for both radioiodination and for standards. Bio-assay of this preparation indicated a LH potency of 3.35 (2.60-4.31) NIH-LH-S1 units per mg (6) and a FSH potency of less than 0.06 NIH-FSH-S1 units per mg(7). This material was radioiodinated‡ to specific activities of 200-300 μc per μg by the method of Greenwood *et al*(8). Separation of antibody-bound LH-^{131}I and free LH-^{131}I was achieved using a second antibody, prepared in rabbits, directed against normal guinea pig serum.

All dilutions of reagents were made in 1% bovine serum albumin in barbital buffer, 0.07 molar, pH 8.6. All reactants were added to 12 × 75 mm plastic tubes. Analyses were done in duplicate and standard dose-response curves were run in all assays. Standards and unknown were made up to a volume of 1.0 ml. Unknown sera were used in a volume of 0.05 to 0.2 ml. One tenth ml of anti-LH serum was added in a concentration (1:5000) which bound 30-50% of the labeled LH. These reactants were incubated for 24 hours at 4°C. One-tenth ml of LH-^{131}I containing 0.1 to 0.2 mμg of protein was then added and incubation was continued for another 24 hours at 4°C.

Fifteen-hundredths ml of rabbit anti-guinea pig serum and 0.1 ml of a 1:200 dilution of carrier normal guinea pig serum were added and allowed to incubate for an additional 72 hours at 4°C. The tubes were then centrifuged at 5000 rpm for 20 minutes. The supernatants and precipitates were counted in an automatic gamma-well counter and the percentage of total counts in each precipitate was calculated.

‡ Using carrier-free sodium iodide-131, Iso-Serve, Cambridge, Mass.

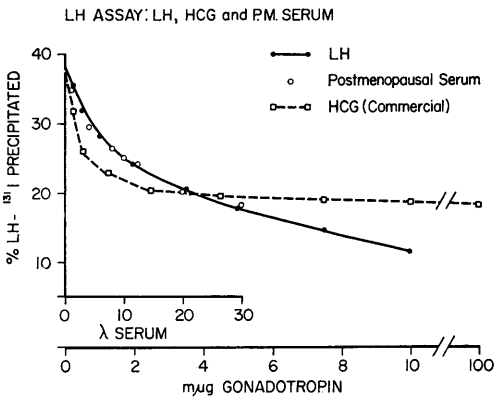

FIG. 1. Plot of dose-response curves for human LH standard, commercial HCG and LH content of post-menopausal serum.

Results and discussion. Over 98% of the LH-^{131}I was precipitable with tungstic acid and up to 90% was immunologically precipitable.

A typical standard curve for LH is shown in Fig. 1. The sensitivity of the assay is such that 0.1-0.2 mμg of standard can be detected.

Pituitary and urinary LH fractions of human origin varying greatly in their LH and FSH biological activities were tested in the radioimmunoassay. Unless otherwise specified the pituitary fractions were prepared in this laboratory. The results are shown in Table I. The index of discrimination for the pituitary preparations is close to unity except for the highly purified FSH fractions 5765B, 5465B and LER-780. There is a 2- to 9-fold overestimation of the LH content in these fractions by the immunologic assay. This does not appear to be nonspecific cross-reactivity with FSH for there was not a consistent overestimation of all the other fractions tested including the highly purified FSH fractions 11867C and R-828-2. Rather, this may represent the selective inactivation of LH biological sites during the purification procedure. The presence of significant amounts of immunologically reactive LH material in the 5765B fraction may explain the apparent partial cross-reactivity observed with LH in

TABLE I. A Comparison of Immunoassay and Bioassay Potency Estimates.*

Fraction	Bio-FSH	Bio-LH (95% CL)	Immuno-LH
Pituitary fractions			
41965B1BA	<.06	2.65 (1.86 –3.78)	1.67
61766B	<.5	2.80 (1.89 –4.14)	2.56
9765B	<.09	4.16 (2.11 –8.20)	6.17
11165ADPP	.08	.006 (.004– .010)	.004
9965B2BC	4.98	.08 (.04 – .16)	.10
5765B	100.0	.079 (.04 – .16)	.70
5465B	103.3	.081 (.05 – .14)	.16
11867C	104.8	.21 (.11 – .41)	.23
LER-780†	53.4	.28	.80
R-828-2†	135 0	.21	.20
Urinary fractions			
R-733-2†	.58	.008	.0029
Pergonal 1990‡	1.44	.033	.014
Pergonal 2074‡	2.70	.14	.045
2nd IRP-HMG§	.31	.0053	.0034

* All results expressed as NIH-S1 units per mg.

† Fractions and bioassay data kindly provided by Dr. L. E. Reichert, Dept. of Biochemistry, Emory Univ., Atlanta, Ga.

‡ Specimens of pergonal (human post-menopausal urinary gonadotropin) and bioassay data on these kindly provided by Mr. C. R. Thompson, Cutter Laboratories, Berkeley, Calif.

§ The Second International Reference Preparation of human menopausal gonadotropin was obtained from the Medical Research Council, Nat. Inst. for Medical Research, Mill Hill, London, England.

our previously described radioimmunoassay procedure for FSH(9) which was made specific for FSH by absorption of the antiserum with HCG.

It can be seen from Table I that there was consistent understimation of the urinary fractions by the immunologic assay. A similar underestimation can also be seen in previously published data(1). This underestimation by immunoassay has also been observed by us for the FSH content of urinary fractions using a pituitary standard(9). These findings suggest the selective inactivation of immunologically reactive sites as a result of metabolism of these hormones or alteration during purification from urine.

Commercial HCG§ was observed to cross-react in an unexpected manner. This is shown in Fig. 1. It can be seen that at concentrations below 4 mμg, HCG is better able to displace labeled LH than is unlabeled LH, whereas the converse is true at higher concentrations. Thus, there is no simple relationship between LH and HCG in this assay system pointing out the potential difficulty of using a single reference standard in such systems. Other antisera prepared in this laboratory against commercial HCG have shown consistently greater sensitivity to HCG than to LH.

§ APL, Ayerst, New York, New York.

Specificity of the assay with regard to other pituitary hormones has not been investigated systematically. The failure of serum from cretinous children¶ to cross-react and the observation (Table I) that crude pituitary extracts are not overestimated by immunoassay appear to support the contention that the assay is specific for LH.

The assay was applied to the measurement of LH in unconcentrated serum. Dilutions of post-menopausal serum gave a dose-response slope parallel to that of the LH standard. This can be seen in Fig. 1. LH added to post-menopausal serum was recovered quantitatively. The results of sera tested thus far are shown in Table II. It should be noted

TABLE II. Serum LH Levels as mμg of Standard per ml.

Subjects	Mean level	Range	n
Normal adult men	2.9	1.5– 4.6	12
" " women*	3.9	2.0– 6.6	13
Hypopituitary adults	1.2	.0– 3.5	8
Prepubertal children	1.6	.4– 2.9	15
Postmenopausal women	12.6	7.8–20.0	6
Primary hypogonadal male	14.6	—	1
Turner's syndrome	19.6	—	1

* Excluding ovulatory peak levels.

that 1 mμg of our LH standard represents 3.35 μU (NIH-LH-S1) and 7.8 mIU of LH (2nd IRP).

Nine women were observed during the course of 11 ovulatory menstrual cycles. A single LH peak was observed at, or near, mid-cycle. The peak levels of 8.3 to 20.3 mμg per ml were 2 to 5 times their pre-ovulatory and post-ovulatory serum concentrations. This cyclic variation has previoulsy been observed (1,10) as has the presence of LH in the serum of prepubertal children(1).

¶ Kindly provided by Dr. R. M. Blizzard, Department of Pediatrics, Johns Hopkins University, Baltimore, Maryland.

The finding of LH in the serum of hypopituitary subjects is of interest and is similar to the situation seen with growth hormone levels in hypopituitarism. Only with suitable stimulative tests can one readily differentiate between the normal state and that of pituitary insufficiency(11). Even with surgical hypophysectomy one might expect to find circulating levels of LH, for LH-like material has been demonstrated by a fluorescent-antibody technique not only in the anterior pituitary but also in the pars tuberalis and the posterior lobe as well(12).

The results presented herein agree well with those of the previously published methods for the radioimmunoassay of LH(1,2) despite certain differences in methodology. These observations suggest that the radioimmunoassay for human LH is a reliable and specific method for quantitating this hormone in serum.

Summary. A sensitive and specific radioimmunoassay technique for quantitating human luteinizing hormone (LH) is described. Purified human pituitary LH is used for the development of antibodies, for radioiodination and for standards. A good correlation was seen between biological and immunological potency estimates of a number of pituitary extracts varying greatly in their LH and FSH contents. This method, when applied to serum, revealed differences in levels between normal adults and postmenopausal women. Cyclic variation was noted in the normally menstruating woman and the presence of LH in the serum of prepubertal children was also confirmed.

The pituitaries used in this study were obtained from the National Pituitary Agency. Biological standards were obtained from the Endocrine Study Section, Nat. Inst. Health. The authors wish to thank Dr. J. Greslin and Miss L. Stevenson for their assistance.

1. Odell, W. D., Ross, G. T., Rayford, P. L., J. Clin. Invest., 1967, v46, 248.
2. Midgley, A. R., Jr., Endocrinology, 1966, v79, 10.
3. Franchimont, P., Ann. Endocrinol., 1966, v27, 273.
4. Bagshawe, K. D., Wilde, C. E., Orr, A. H., Lancet, 1966, v1, 1118.
5. Wide, L., Roos, P., Gemzell, C., Acta Endocrinol., 1961, v37, 445.
6. Parlow, A. F., *In* Albert, A., ed., Human Pituitary Gonadotropins, Charles C Thomas, Springfield, Ill., 1961, p300.
7. Steelman, S. L., Pohley, F. M., Endocrinology, 1953, v53, 604.
8. Greenwood, F. C., Hunter, W. M., Glover, J. S., Biochem. J., 1963, v89, 114.
9. Faiman, C., Ryan, R. J., J. Clin. Endocrinol., 1967, v27, 444.
10. Midgley, A. R., Jr., Jaffe, R. B., ibid., 1966, v26, 1375.
11. Roth, J., Glick, S. M., Yalow, R. S., Berson, S. A., Science, 1963, v140, 987.
12. Midgley, A. R., Jr., Exp. Cell Res., 1963, v32, 606.

Received April 10, 1967. P.S.E.B.M., 1967, v125.

ATP Content of Spermatozoa, Semen, and Seminal Plasma. (32296)

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The firefly assay has been employed for quantitative estimation of ATP in spermatozoa, whole semen, and seminal plasma. The intensity of the light reaction of the firefly *(Photinus pyralis* or *Photurus pennsylvanicus)* which is mediated by the enzyme luciferase depends on the amount of luciferin and ATP present(1,2). ATP can be quantitatively assayed by measuring the light intensity which follows addition of ATP to a luciferin-luciferase preparation(3,4).

Materials and methods. Six to twelve firefly lanterns (Sigma Chemical Co.) were used for each ml of crude luciferin plus luciferase desired. Luciferin and luciferase were ex-

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