Localization of Radioactivity in Immature Rat Ovaries Following Physiological Doses of ^{125}I-Labeled Bovine LH[1] (36050)

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(Introduced by R. O. Greep)

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The plasma half-life in the rat of exogenous pituitary LH from a variety of species has been shown to be relatively short (1, 2). With the exception of equine LH, reported values ranged from 10 to 65 min. A rapid plasma disappearance has recently been confirmed for endogenous rat gonadotropin immediately following hypophysectomy (3).

Parlow (4) attempted to measure LH activity in urine following a single intravenous injection of 1 mg (NIH-LH-S1) to adult female rats. No detectable LH activity, however, was recoverable in urine collected for 12 hr following injection (<4 μg/12 hr). Kohler *et al.* (5) suggested that reported values for urinary excretion of LH in the human female measured by bioassay account for only a fraction of that produced by the pituitary per unit time.

Although disappearance of LH from plasma is rapid and little pituitary activity appears unchanged in urine, the site of the rapid metabolism of circulating LH has not been established. Neither is it clear whether gonadotropin accumulates in target tissue or whether target tissue plays a role in the metabolism of this hormone.

In the present study, bovine LH was labeled with ^{125}I and evaluated for immunological activity. A dose of ^{125}I-labeled LH within the physiological range was administered to immature female rats and the distribution of label was followed *in vivo* from 5 min to 12 hr.

Methods and Materials. Labeling procedure. Five micrograms of bovine LH (NIH-LH-B4) were labeled with 1 mCi of ^{125}I ($Na^{125}I$, Iso-Serve Inc., Cambridge, MA), using the chloramine T oxidation method of Greenwood *et al.* (6). Following the iodination reaction, 50 μl of human serum were added, and the reaction mixture was subjected to gel filtration on a column of Sephadex G-100 prepared in a 10-ml blow-out pipet (bed volume = approx 5 ml). The column was equilibrated and eluted with 0.05 *M* NH_4HCO_3 buffer, pH 8.0. One-half milliliter fractions were collected manually and counted on a Nuclear Chicago gamma counter at a fixed geometry of 1 ft. Elution volume (V_e) of radioactivity peaks was expressed as the ratio, elution volume/column bed volume (V_e/V_c).

Immunological characterization of labeled LH. For the determination of the immunological activity of ^{125}I-LH, the three fractions representing peak protein radioactivity, as eluted from Sephadex following iodination, were pooled for reaction with antiserum to bovine LH. Two 0.7-ml aliquots of the pooled fractions of ^{125}I-LH in NH_4HCO_3 buffer were taken, one for incubation with 0.1 ml of neutral horse serum and one for incubation with 0.1 ml of horse antiserum to bovine LH (0.1 ml neutralized 80 μg of LH activity *in vivo* in immature rats). Incubations were carried out for 30 min at 37° followed by overnight at 4°.

^{125}I-LH:anti-LH complex was separated from smaller molecules by subjecting aliquots

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of the ^{125}I-LH:serum mixtures to either gel filtration on Sephadex G-200 or to double antibody precipitation (7) using rabbit antiserum to horse gamma-globulin (anti-HGG) (Hyland Laboratories, Los Angeles, CA).

Gel filtration on Sephadex G-200 employed two identically prepared 0.6 × 30 cm columns, each loaded prior to use with 1.0 ml of 2% bovine serum albumin (fraction V) and washed through with 20 ml of 0.05 *M* NH_4HCO_3 buffer according to the procedure of Greenwood *et al.* (6) for gel filtration of protein following iodination. All equilibration of gel and elution from columns was done with 0.05 *M* NH_4HCO_3 buffer. One milliliter of ^{125}I-LH:normal serum mixture and of ^{125}I-LH:anti-LH serum mixture were each added to a column. Fractions were collected and counted for ^{125}I radioactivity as described above.

For double antibody precipitation of ^{125}I-LH:anti-LH complex, 100 μl of a 1:200 dilution of each ^{125}I-LH:horse serum mixture was incubated for 1 day at 4° with 300 μl of anti-HGG, 100 μl of 0.1 *M* EDTA, and 500 μl of 1% bovine serum albumin in 0.05 *M* NH_4HCO_3 buffer. Following incubation, samples were centrifuged in the cold at 700*g* for 20 min. Supernatant liquids and precipitates were counted in a Nuclear Chicago automatic well-type gamma counter for ^{125}I radioactivity.

In vivo *distribution of ^{125}I-LH.* For injection into immature female rats, three fractions representing peak protein radioactivity, as collected from Sephadex G-100 following iodination, were again pooled. A 1.2-ml aliquot from this pool was diluted to 18 ml with 0.9% NaCl. Forty 21-day-old female Holtzmann rats were injected iv into the tail vein under light ether anesthesia with 51.1 ng (7.68 μCi) of ^{125}I-LH/100 g of body weight.

Animals were killed in eight groups, 5 rats per group, at the following times after injection: 5, 15, and 30 min; 1, 2, 4, 6, and 12 hr. Animals were killed by decapitation following chloroform anesthesia, and blood was collected from the severed carotid arteries through a funnel into a centrifuge tube. Serum was separated by centrifugation and a 0.25-ml aliquot was taken for assay of ^{125}I radioactivity. Ovaries, adrenals, thyroids, and a portion of liver were removed and weighed to the nearest 0.5 mg, and transferred directly to test tubes for radioassay of ^{125}I. Radioactivity was measured on a Nuclear Chicago automatic well-type gamma counter. Tissue radioactivity was expressed as disintegrations per minute per milligram tissue wet weight or per microliter of serum.

Results. Iodination. Specific activities for ^{125}I-LH, calculated as described by Greenwood *et al.* (6), ranged from 150 to 176 μCi/μg. The calculated ratio for atoms of ^{125}I per molecule of bovine LH (estimated mol wt = 30,000) (8) ranged from 2.1 to 2.4.

Characterization of labeled LH. The elution patterns from Sephadex G-200 of ^{125}I-LH:horse serum mixtures are shown in Fig.

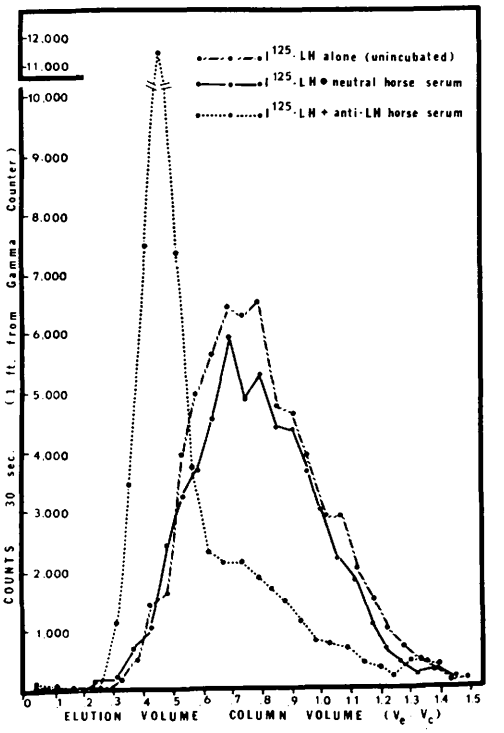

FIG. 1. Elution pattern of ^{125}I radioactivity following gel filtration of ^{125}I-IH and ^{125}I-LH:horse serum mixtures on 3 identical columns of Sephadex G-200. The peak eluted from gel ahead of unincubated ^{125}I-LH is presumed to be antibody-bound ^{125}I-LH. The peak eluted in the same region as unincubated ^{125}I-LH is presumed to be nonbound ^{125}I-LH.

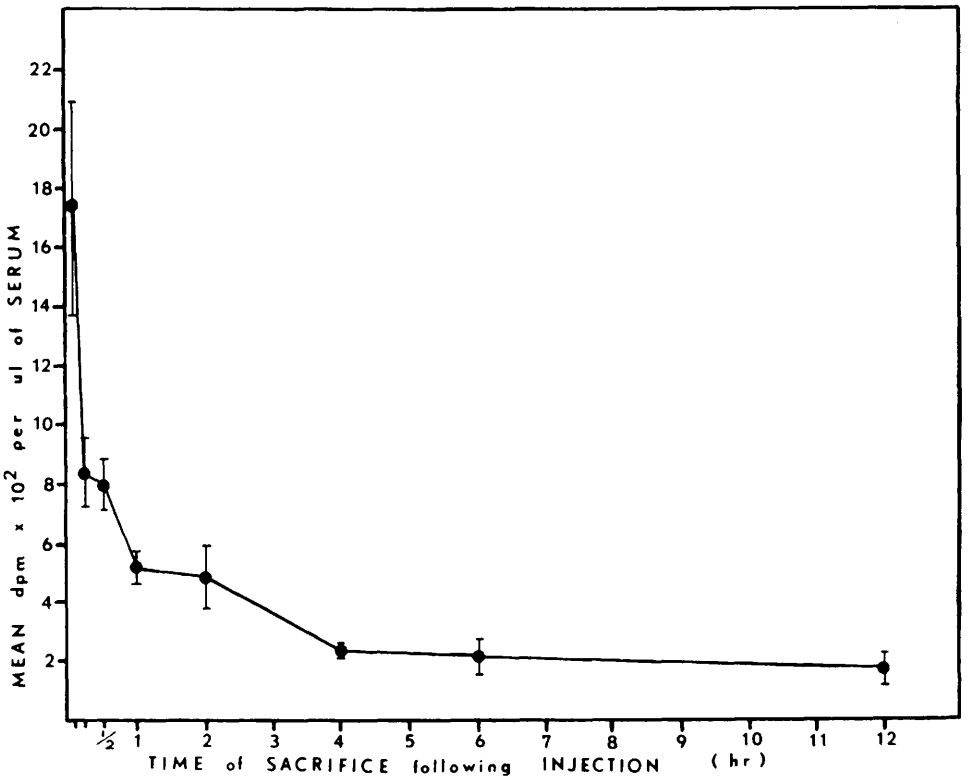

FIG. 2. Disappearance of ^{125}I radioactivity from serum following iv administration of 51.1 ng (7.68 μCi) of ^{125}I-LH/100 g of body weight to immature female rats. Five animals were sacrificed at each of the following time periods after injection: 5, 15, and 30 min; 1, 2, 4, 6, and 12 hr. Each point represents the mean serum concentration of ^{125}I radioactivity for 5 animals. Standard deviations are indicated by brackets.

1. ^{125}I-LH incubated with neutral serum gave a wide protein peak on elution from G-200 with V_e/V_c = 0.695 — 0.802. For comparison, a nonincubated aliquot of ^{125}I-LH alone eluted from G-200 gave the same elution pattern (V_e/V_c = 0.699 — 0.806) as the ^{125}I-LH:neutral horse serum mixture (Fig. 1). In contrast, ^{125}I-LH incubated with anti-LH horse serum, on elution from G-200, gave a sharp early peak at V_e/V_c = 0.469 equivalent to 71% of the total radioactivity recovered, and only a small shoulder (V_e/V_c = 0.677 — 0.937) corresponding to the wide protein peak apparently characteristic of ^{125}I-LH.

Radioassay of supernatant liquids and precipitates following double antibody precipitation of ^{125}I-LH:anti-LH serum revealed that 64% of the total radioactivity was precipitable as antibody-bound.

In vivo *distribution of ^{125}I-LH*. Disappearance of radioactivity from blood following intravenous injection of ^{125}I-LH to immature female rats is plotted in Fig. 2 (mean dpm/μl of serum vs time of sacrifice following injection). Disappearance rate from 5 min to 12 hr approximates a simple hyperbolic function (9) with a calculated half-life of 19.6 min. In view of the report of Hutchinson *et al.* (10) suggesting that bovine LH disappears from rat plasma at a rapid rate similar to ovine LH, the present result is in good agreement with the reported plasma half-life values for ovine LH in intact rats throughout the cycle [15 min, Parlow (1); 22.3 min, Eder and Lipner (2)] and for endogenous LH following acute hypophysectomy in proestrous rats [20 min, Gay *et al.* (3)].

^{125}I radioactivity in ovary, adrenal, liver, and thyroid is plotted in Fig. 3a (mean dpm/mg of tissue wet weight vs time of sacrifice

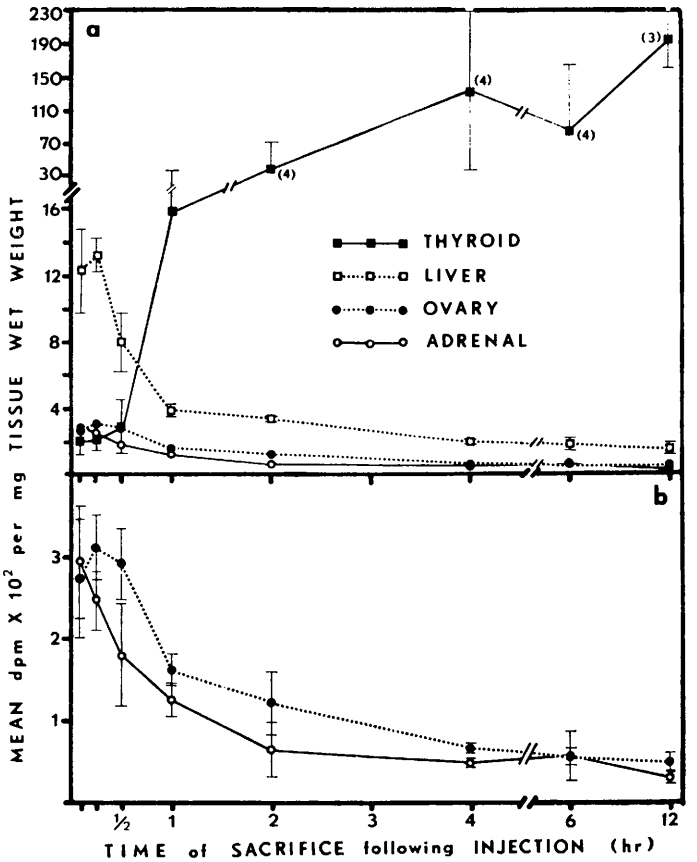

FIG. 3. (a) ^{125}I radioactivity in thyroid, liver, ovary, and adrenal at intervals from 5 min to 12 hr following iv administration of 51.1 ng (7.68 μCi) of ^{125}I-LH/100 g of body weight to immature female rats. Each point represents the mean tissue concentration of radioactivity for 5 animals except where the number of animals is indicated in parentheses. Standard deviations are indicated by brackets. (b) Radioactivity in ovary and adrenal is replotted from (a) using an expanded scale.

following injection of ^{125}I-LH). The tissue accumulating the highest level of radioactivity was thyroid, but thyroid radioactivity remained low through 30 min following injection, increased rapidly from 1 to 2 hours, and maintained its high level through 12 hr following injection. Reported uptake (11) by rat thyroid of free ^{131}iodide (% of injected dose/g of tissue) by 1 min following intravenous injection was comparable to the level of thyroid radioactivity reached in the present experiment by 1–2 hr. Since free iodide is concentrated so rapidly in thyroid, the curve described in Fig. 3a indicates that, in the present experiments, little free iodide was present in the injected material. It is likely then that ^{125}I radioactivity was concentrated in thyroid as free ^{125}iodide only after iodine atoms were split from protein. It is obvious, however, from the delayed but massive uptake by thyroid, that free iodide was released rapidly *in vivo* following injection of the iodinated protein.

Accumulation of radioactivity in liver reached a higher level per milligram than in ovary and adrenal (Fig. 3a). Radioactivity in liver increased from 5 min to a maximum at 15 min, then decreased, apparently exponentially, from 15 min to 12 hr. This 15 min maximum uptake of radioactivity by liver is similar to that reported by Sonenberg *et al.* (11) following intravenous injection of ^{131}I-labeled ACTH to rats and by Berson *et al.* (12) following intravenous ^{131}I-labeled

human globin to humans. Berson *et al.* (12) suggest that this time sequence of radioactivity uptake in liver represents localization of protein in liver, followed by proteolysis, release of iodinated tyrosine residues, and subsequent rapid metabolism of amino acids to release free ^{131}iodide. In the present experiment, the levels of radioactivity in liver at 15 min represent 16% of the total injected dose per gram of tissue, or [using 2.7 g as the value for organ weights in these animals (13)] an estimated 43% of injected dose per whole organ. This result might suggest a similar route via liver for the metabolism of LH as that described by Berson *et al.* (12) for globin.

Radioactivity appeared in ovary and adrenal at similar low levels (Fig. 3a and b) representing, at the early time periods, only 0.05–0.07% of injected dose per pair of glands. Level of radioactivity in adrenal declined, apparently exponentially, from a maximum at 5 min, the earliest time period measured. In contrast, radioactivity increased in ovary from 5 to 15 min following injection, remained elevated at 30 min, and then declined exponentially from that time period. Analysis of variance performed on the data from ovary and adrenal across all time periods indicated, by a significant interaction ($p<.05$) of time $\times$ tissue effects, that accumulation and loss of radioactivity by these two tissues is represented by different functions. Figure 3b indicates that these differences occur at the early time periods. Ovary appeared to retain label up to 30 min, while adrenal did not.

Discussion. The plasma half-life value of 19.6 min for physiological levels of LH reported in the present experiment is in good agreement with the rapid plasma disappearance of LH reported for a variety of experimental conditions (1–3). The somewhat longer half-life (19–38 min) reported by Gay and Bogdanove (14) for endogenous LH in castrated rats might reflect the absence of ovarian tissue accumulating LH. Although the disappearance function of radioactivity from plasma (Fig. 2) in the present experiment might be complicated by the disappearance of free ^{125}iodide from plasma, this should not be expected to interfere with the calculated disappearance rate at the very early time periods which are of interest in reference to LH. Uptake of radioactivity into thyroid indicates the presence of little free iodide before 1–2 hr following injection.

The data representing tissue levels of radioactivity in the present experiment suggest two possible sites for the accumulation of LH. Accumulation of 43% of the total injected dose of ^{125}I-LH in liver at a 15-min maximum level following injection might suggest that LH is metabolized by proteolysis in the manner described by Berson *et al.* (12) for human globin. The high proportion of injected dose accumulating in liver would argue that at least some of this radioactivity represents LH protein. The question arises whether LH in this case is treated as a nonspecific foreign protein by liver and whether such accumulation would occur following normal physiological secretion from the pituitary. Gay and Bogdanove (14) have demonstrated no measurable difference in the plasma disappearance rate of exogenous ovine LH or endogenous rat LH in the castrate rat, which would indicate no unique route of metabolism for the exogenous hormone. The present results suggest that liver may play a role in the deactivation of circulating LH.

Several investigators have demonstrated the uptake of iodine-labeled HCG by ovaries (15, 16). In the present study, the minimal concentration of ^{125}I-LH by the ovaries is consistent with the small, but significant, difference between LH levels in arterial and ovarian venous plasma in women found by Llerena *et al.* (17). Immunological and biological characterization of the radioactivity retained by the ovaries in the present study should reveal whether this accumulation of label represents a physiologically significant hormone–target tissue interaction.

Summary. Bovine LH was labeled with ^{125}I by chloramine T oxidation and gel filtration. Immunological characterization of iodinated LH, using either gel filtration on Sephadex G-200 or double antibody precipitation to separate free and antibody-bound ^{125}I-LH, indicated that 71 and 64%, respectively, of total radioactivity was reactable

with antibody to bovine LH.

A dose of ^{125}I-LH within the physiological range (51 ng/100 g of body wt) was administered intravenously to immature female rats. Serum samples, ovaries, adrenals, thyroids, and portions of liver were taken for radioassay at times ranging from 5 min to 12 hr following injection. Disappearance of radioactivity from blood approximated a hyperbolic function with a half-life of 19.6 min. Radioactivity in liver at its 15-min maximum represented 43% of the total injected dose per whole organ. Radioactivity in ovary and adrenal appeared at similar low levels (0.05–0.07% of injected dose per pair of glands). Disappearance curves from the two organs, however, were significantly different, with radioactivity in ovary, but not in adrenal, being retained up to 30 min following injection.

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1. Parlow, A. F., Program Endocrine Soc. 43rd Meet., New York, p. 8 (1961).

2. Eder, D. J., and Lipner, H., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **29**, 440 (1970).

3. Gay, V. L., Midgley, A. R., Jr., and Niswender, G. D., Fed Proc., Fed. Amer. Soc. Exp. Biol. **29**, 1880 (1970).

4. Parlow, A. F., Endocrinology **74**, 298 (1964).

5. Kohler, P. O., Ross, G. T., Tullner, W. W., and Odell, W. D., J. Clin. Invest. **46**, 1079 (1967).

6. Greenwood, F. C., Hunter, W. M., and Glover, J. S., Biochem. J. **89**, 114 (1963).

7. Feinberg, R., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **13**, 493 (1954).

8. Reichert, L. E., Jr., and Jiang, N. S., Endocrinology **77**, 78 (1965).

9. Lewis, D., "Quantitative Methods in Psychology," p. 51. McGraw-Hill, New York (1960).

10. Hutchinson, J. S. M., Armstrong, D. T., and Greep, R. O., J. Endocrinol. **40**, 231 (1968).

11. Sonenberg, M., Keston, A. S., and Money, W. L., Endocrinology **48**, 148 (1951).

12. Berson, S. A., Yalow, R. S., Post, J., Wisham, L. H., Newerly, K. N., Villazon, M. J., and Vasquez, O. N., J. Clin. Invest. **32**, 22 (1953).

13. Fukuda, M., and Sibatani, A., J. Biochem. (Tokyo) **40**, 95 (1953).

14. Gay, V. L., and Bogdanove, E. M., Endocrinology **82**, 359 (1968).

15. Eshkol, A., and Lunenfeld, B., *in* "Gonadotropins 1968" (E. Rosemberg, ed.), p. 187. Geron-X, Los Altos, CA (1968).

16. Espeland, D. H., Naftolin, F., and Paulsen, C. A., *in* "Gonadotropins 1968" (E. Rosemberg, ed.), p. 177. Geron-X, Los Altos, CA (1968).

17. Llerena, L. A., Guevara, A., Lobotsky, J., Lloyd, C. W., Weisz, J., Pupkin, M., Zanartu, J., and Puga, J., J. Clin. Endocrinol. Metab. **29**, 1083 (1969).

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