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DISAPPEARANCE AND UPTAKE OF [¹²⁵I]FSH IN THE RAT, RABBIT, EWE AND COW*

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Summary. Follicle-stimulating hormone (NIH-FSH-s8) was labelled with ¹²⁵I to determine its disappearance rate after a single intravenous injection and to determine the level of circulating [¹²⁵I]FSH in the blood after a single intramuscular or subcutaneous injection in the rat, rabbit, ewe and cow. There was a difference in the disappearance and uptake rates among the four species, but the shape of the curve for rate of loss and uptake of labelled FSH was similar in all species. The disappearance of radioactivity occurred at two rates; the first from 1 to 8 min and the second from 16 to 96 min. The half-life, calculated from the total decay curve in each species was 94 ± 21 , 118 ± 16 , 334 ± 41 and 301 ± 23 min for the rats, rabbits, ewes and cows, respectively. Intramuscular injections resulted in an average of 56% higher [¹²⁵I]FSH blood levels than subcutaneous injections for all species.

INTRODUCTION

It is essential to know the fate of injected preparations of FSH, as well as the endogenous levels of this gonadotrophin, to evaluate its effects upon the growth and maturation of ovarian follicles. The labelling of FSH with a radioactive isotope that does not destroy the biological activity of the hormone offers a promising method for monitoring disappearance and uptake rates of this gonadotrophin. Follicle-stimulating hormone has been labelled with radioactive iodine to determine turnover rates from endogenous and exogenous sources in man and rats (Bogdanove & Gay, 1969; Coble, Kohler, Cargille & Ross, 1969; Cons & Kragt, 1970; Gay, Midgley & Niswender, 1970; Yen, Llerena, Pearson & Littell, 1970; Gay, 1971).

This investigation deals with the disappearance and uptake rates of exogenous FSH in different species. The study was designed to compare disappearance of FSH given intravenously (i.v.) and its uptake when injected intramuscularly (i.m.) or subcutaneously (s.c.) in the rat, rabbit, ewe and cow.

MATERIALS AND METHODS

Radioiodination

Radioiodination of NIH-FSH-s8 was carried out with ¹²⁵I at room temperature

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by a modification of the method of Greenwood, Hunter & Glover (1963). The specific activity of the ^{125}I was $200 \mu\text{Ci}/\mu\text{g}$. Chloramine-T ($5 \mu\text{g}$) was added to $2.5 \mu\text{g}$ hormone and the reaction was stopped after 30 sec by adding $125 \mu\text{g}$ sodium metabisulphite in $50 \mu\text{l}$ 0.5 M -phosphate buffer, pH 7.5. Immediately after iodination, the reaction mixture was separated with a Bio-Gel P 100 column ($40 \text{ cm} \times 1.0 \text{ cm}$). Fractions (1 ml) of the column eluate were collected in tubes containing 1 ml 0.05 M -phosphate buffer with 5% freeze-dried egg white, pH 7.5. The labelled hormone was used within 2 days of iodination.

Biological assays and gel filtration

Location of the labelled eluant containing follicle-stimulating activity was determined by isolating the fraction containing the labelled hormone and assaying its biological activity. Fractions from the three peaks in the elution of the iodinated preparation were mixed with 2.0 mg unlabelled NIH-FSH-s8 and filtered through a Bio-Gel P-150 column ($40 \text{ cm} \times 1.5 \text{ cm}$). It was assumed that the labelled FSH, which was biologically active, and the unlabelled FSH would mix homogeneously and be eluted from the column at the same time.

Radioactivity from this column was eluted in three peaks and the eluants from each peak were assayed for FSH activity by the HCG augmentation method (Steelman & Pohley, 1953). Protein in segments of the labelled-unlabelled preparation elution curve were determined by the procedure of Lowry, Rosebrough, Farr & Randall (1951).

Relative biological potency of the labelled preparation was estimated by using a 'cold-labelled' FSH preparation in the HCG augmentation method of Steelman & Pohley (1953). The NIH-FSH-s8 ($1.62 \mu\text{g}$) was subjected to similar amounts of sodium iodide, chloramine-T and the other reagents used in the radioactive labelling procedure before injection into the assay rats.

The following procedures were used to determine if the ^{125}I was attached to the FSH molecule at various intervals after injecting [^{125}I]FSH into the animals. Labelled FSH, mixed with 0.3 ml serum, and ^{125}I , mixed with 0.3 ml serum, was passed through a Bio-Gel P-150 column ($40 \text{ cm} \times 1.5 \text{ cm}$) at different times and eluted in 2-ml fractions. The fractions were counted in a gamma-well scintillation counter for 5 min. Serum (0.3 ml) taken from a cow 8, 64, 96 or 256 min after a single i.v. injection of [^{125}I]FSH was passed through the same column and collected in 2-ml fractions. Labelled FSH, mixed with dextran 2000, was injected i.m. or s.c. into rats and these sites of injection were dissected 128 min later. The tissue was placed in 2 ml 0.05 M -phosphate buffer, pH 7.5. The liquid fraction was aspirated 18 hr later and 0.3 ml was passed through the column used for the standard [^{125}I]FSH and ^{125}I preparations.

Animals and treatments

Disappearance rates of [^{125}I]FSH after a single i.v. injection and uptake rates after a single i.m. or s.c. injection were determined in the rat, rabbit, ewe and cow. The specific radioactivity per injection was approximately $50 \mu\text{Ci}$ for the rats, approximately $100 \mu\text{Ci}$ for the rabbits and 150 to $200 \mu\text{Ci}$ for the ewes and cows. Sites of the injections were the tail vein of the rats, the femoral vein of the rabbits and jugular vein of the ewes and cows, the pectoral

muscle and s.c. into the hind leg of each species. At each time interval, a 0.5-ml blood sample was taken from the rats and rabbits and a 1-ml blood sample from the ewes and cows.

Two-hundred cycling, female Holtzman rats were used. Disappearance rates of labelled FSH after i.v. injection and uptake rates after i.m. injection were determined in 160 rats during the oestrous (1st day of cornified vaginal smears) and dioestrous (2nd day of leucocyte vaginal smears) phases of a 5-day oestrous cycle and 2 or 21 days after bilateral ovariectomy. The rats were ovariectomized on the 2nd day of dioestrus. Uptake rates after i.m. or s.c. injections were determined during the dioestrous phase of the cycle in forty rats. Blood samples were taken 1, 8, 16 and 64 min after the i.v. injections and 16, 32, 64 and 128 min after the i.m. and s.c. injections. Only two blood samples were taken from each rat by cardiac puncture.

Twelve Dutch Belted rabbits were used; four injected i.v., four i.m. and four s.c. The jugular vein was cannulated and blood samples were collected 1, 2, 4, 8, 16, 32, 48, 64, 80, 96, 112 and 128 min after injection of the labelled hormone.

Three cycling crossbred ewes and three cycling Holstein heifers were used in separate reversal experiments. Each animal was injected i.v. with the labelled hormone during the luteal (Day 8) and follicular (Day 17) phases of the oestrous cycle and i.m. or s.c. only during the follicular phase of the cycle. The jugular vein was cannulated and blood samples were obtained 1, 2, 4, 8, 16, 32, 48, 64, 96, 128, 160, 192, 224 and 256 min after injection of the [125 I]FSH preparation.

The half-life ($T_{1/2}$) was calculated by multiplying the slope of the regression curve ($Y = \log_{10} \text{ct/min}$; $X = \text{time}$) by 2.303 and dividing this product into 0.693 (Wang & Willis, 1965).

Statistical analyses

The data were analysed by analysis of variance with treatment means obtained by least squares analysis. Data from the ewes and cows were analysed as a split-plot design, from the rabbits as a completely randomized design and from the rats as a randomized block design with rats as blocks across time. Orthogonal comparisons of disappearance and uptake rates were made for the rabbit, ewe and cow data (Snedecor, 1956).

RESULTS

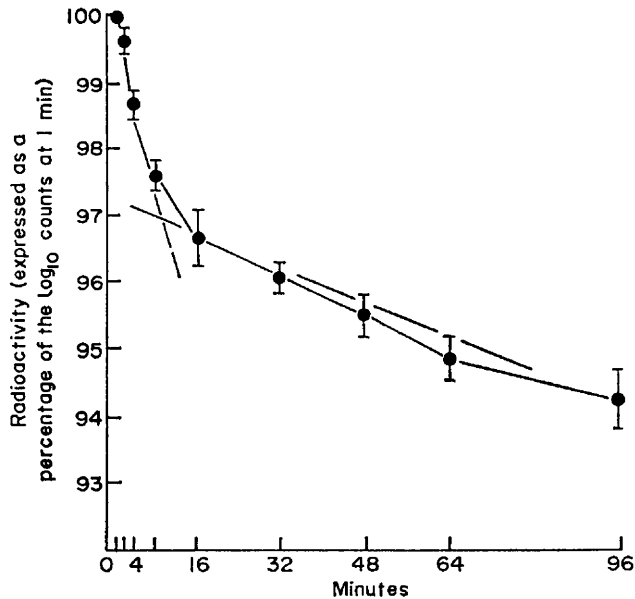
Biological assays and gel filtration

The labelled FSH was found in the first peak of the elution curve after separation of the reaction mixture with Bio-Gel P-100. Elution fractions from the first peak of the labelled-unlabelled hormone preparations stimulated ($P < 0.01$) an increase in ovarian weight in the HCG-augmentation test rats as compared with the HCG controls. Fractions from other segments of the elution curve did not stimulate an ovarian weight increase. The first peak of the elution curve had a greater ($P < 0.05$) amount of protein than the other segments of the elution curve.

Relative biological potency of labelled FSH ($10 \mu\text{g}$ chloramine-T/2.5 mg FSH

for 30 sec) was 0.83 of unlabelled FSH. Larger amounts of chloramine-T for 30 sec resulted in relative biological potencies of less than 0.40 of unlabelled FSH. A reaction time of 2 min also reduced relative potency to less than 0.40.

All the ^{125}I was attached to the FSH molecule in serum taken 8 min after an i.v. injection of ^{125}I FSH, but only 60% of it was bound to the FSH molecule at 64, 96 or 128 min after an i.v. injection of labelled FSH. Gel-filtration (Bio-Gen P-150) of the control ^{125}I FSH and ^{125}I preparations resulted in recovery of 96% of the ^{125}I FSH preparation in the first nine 2-ml fractions and 97% of the ^{125}I preparation in the 11th to 16th fractions. Of the radioactivity in serum taken 8 min after i.v. injections of ^{125}I FSH, 97% was eluted in the first nine 2-ml fractions, while only 60% of the radioactivity in serum taken at 64, 96 or 128 min was eluted in the first nine fractions and 35% in the 11th to 16th fractions.



TEXT-FIG. 1. Disappearance of radioactivity in rats, rabbits, ewes and cows after a single intravenous injection of ^{125}I FSH.

Most of the radioactive iodine was bound to the unabsorbed ^{125}I FSH 128 min after an i.m. or s.c. injection. Of the radioactivity in the tissue extracts taken 128 min after i.m. or s.c. injection of ^{125}I FSH, 93% was eluted in the first nine 2-ml fractions.

Disappearance of ^{125}I FSH

Disappearance of the labelled FSH was similar in all species when the values within each species were expressed as a percentage of the counts/min at 1 min (Text-fig. 1). The disappearance of radioactivity, when plotted on logarithm of counts/min versus time coordinates, had two linear decay rates; the first from 1 to 8 min and the second from 16 to 96 min. The decay rate was faster ($P < 0.01$)

from 1 to 8 min than from 16 to 96 min and faster ($P < 0.01$) from 16 to 96 min than from 96 to 256 min in the ewe and cow. The half-life of the hormone, calculated from different segments of the decay curve for each species, is shown in Table 1. When calculated for each species, the half-life of FSH from the total

TABLE 1
DISAPPEARANCE RATE OF [¹²⁵I]FSH IN THE RAT, RABBIT,
EWE AND COW CALCULATED FROM DIFFERENT SEGMENTS
OF THE DECAY CURVE

Species	$T_{\frac{1}{2}}$ (min)		
	1 to 8 min	16 to 96 min	Total curve*
Rat†	17 ± 4	143 ± 23§	91 ± 21
Rabbit	19 ± 3	143 ± 18	118 ± 16
Ewe†	23 ± 2	239 ± 23	334 ± 41
Cow†	22 ± 1	201 ± 19	301 ± 23

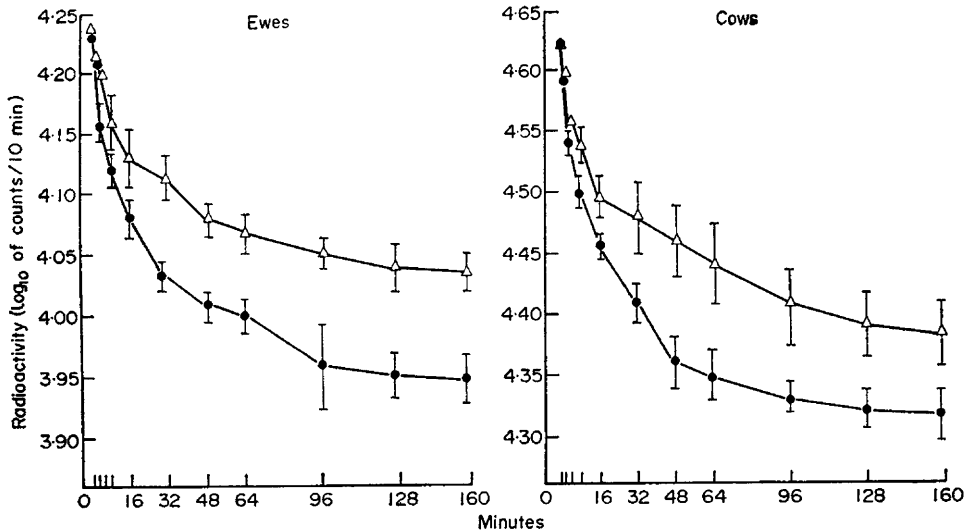
Values expressed in minutes as Mean ± S.E.

* The time for the total decay curve was 1 to 64 min for the rats, 1 to 128 min for the rabbits and 1 to 256 min for the ewes and cows.

† During the oestrous and dioestrous phases of the oestrous cycle.

‡ The mean for the luteal and follicular phases of the oestrous cycle.

§ Time was 8 to 64 min for the rat.

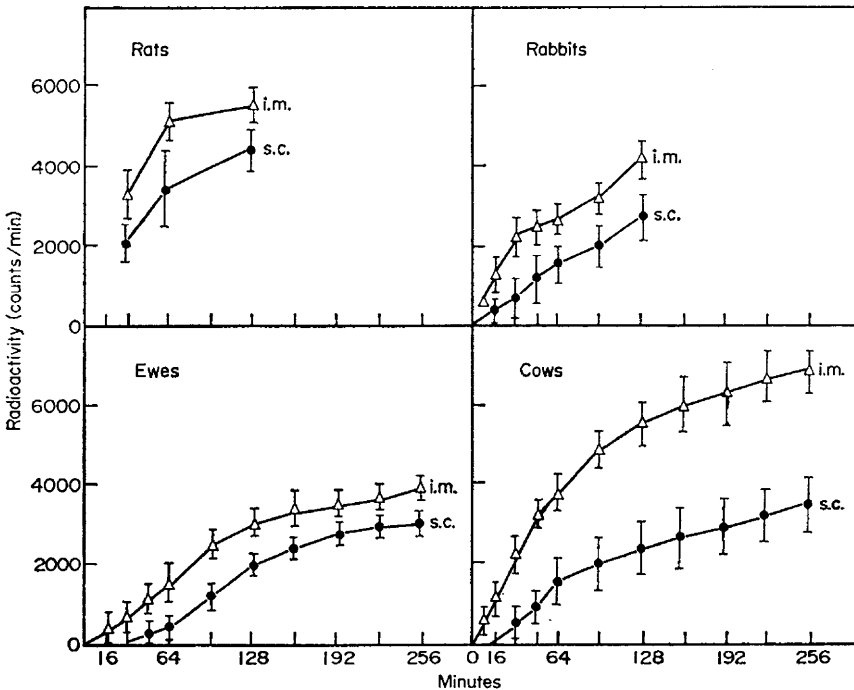


TEXT-FIG. 2. Disappearance of radioactivity after a single intravenous injection of [¹²⁵I]FSH during the follicular (●) and luteal (Δ) phases of the oestrous cycle in ewes and cows.

decay curve was longer ($P < 0.01$) in cattle and sheep than in rats and rabbits. Most of the variation in response from 1 to 128 min in the rabbits and from 1 to 256 min in the ewes and cows was attributable ($P < 0.005$) to the linear and quadratic reductions.

The disappearance rate was faster ($P < 0.01$) during the follicular than during the luteal phase of the oestrous cycle in the ewe and cow (Text-fig. 2). The half-life calculated from the total decay curve (1 to 256 min) was 268 ± 18 min in ewes and 252 ± 27 min in cows during the follicular phase, compared with 393 ± 15 min in ewes and 299 ± 21 min in cows during the luteal phase.

The turnover rate was shorter ($P < 0.01$) in rats injected with labelled FSH during oestrus, dioestrus or 2 days after bilateral ovariectomy than in rats injected 21 days after bilateral ovariectomy. The half-life of labelled FSH calculated from the regression of values at 1, 8, 16 and 64 min was 91 ± 21 min for rats given [125 I]FSH during the oestrous or dioestrous phases of the cycle or



TEXT-FIG. 3. Uptake of radioactivity in rats, rabbits, ewes and cows after a single intramuscular (i.m.) or subcutaneous (s.c.) injection of [125 I]FSH.

2 days after ovariectomy, as compared with 213 ± 23 min for those given [125 I]FSH 21 days after ovariectomy. Disappearance rates were similar among groups of rats treated during oestrus, dioestrus or 2 days after ovariectomy.

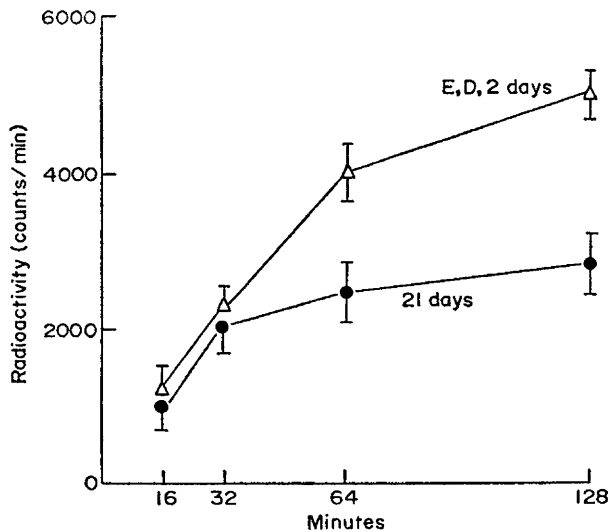
Uptake of [125 I]FSH

The pattern of uptake of labelled FSH into the systemic circulation after a single i.m. or s.c. injection was similar in all species (Text-fig. 3). The uptake rate was quadratic ($P < 0.005$) in the rabbits, ewes and cows. There was some indication for a cubic response ($P < 0.05$) in uptake after an i.m. injection in the rabbits and ewes.

The pattern of hormone uptake was similar after i.m. or s.c. injections in each species (Text-fig. 3). In all species, i.m. injections resulted in faster ($P < 0.01$)

uptake rates than s.c. injections. Intramuscular injections resulted in an average of 56% higher [125 I]FSH blood levels than s.c. injections for all species. The increases were 57% in the rat, 52% in the rabbit, 68% in the ewe and 46% in the cow.

Rats ovariectomized 21 days before receiving an i.m. injection of labelled FSH had lower ($P < 0.01$) blood levels of [125 I]FSH at 64 and 128 min than those given labelled FSH during oestrus, dioestrus or 2 days after ovariectomy (Text-fig. 4). There was no difference in response among those treated during the oestrous or dioestrous phases of the cycle or 2 days after ovariectomy.



TEXT-FIG. 4. Uptake of radioactivity in rats after a single intramuscular injection of [125 I]FSH. Results from rats in oestrus (E), dioestrus (D) and 2 days after ovariectomy (Δ) were similar and were combined, while those from animals 21 days after ovariectomy (\bullet) are shown separately.

DISCUSSION

The decay of [125 I]FSH in the rat, rabbit, ewe and cow in this investigation was similar to that described for endogenous FSH (Coble *et al.*, 1969; Yen *et al.*, 1970), LH (Kohler, Ross & Odell, 1968) and growth hormone (Frohman & Bernardis, 1970) in man and rats. The half-life of FSH for rats, calculated from the total decay curve (91 ± 21 min), was shorter than the value reported for exogenous FSH in female rats (135 min) by Cons & Kragt (1970), but when calculated from values at 8, 16 and 64 min, it was similar (143 ± 23 min).

Half-life values were calculated for the two linear components of the decay curve because both the values and slopes for these components were significantly different ($P < 0.01$) in all species. It has been suggested that this response decreased the importance of $T_{\frac{1}{2}}$ calculated from the early segment of the decay curve (Frohman & Bernardis, 1970). Coble *et al.* (1969) reported that this decay is due to a differential distribution of the exogenous hormone by different organs

in the animal. The increase in half-life of exogenous FSH in nephrectomized rats over controls (Gay, 1971) supports this explanation. Therefore, $T_{\frac{1}{2}}$ values calculated from the total curve more accurately reflect the biological half-life of exogenous FSH than $T_{\frac{1}{2}}$ calculated separately for the first and second linear parts of the decay curve.

If the separation of the ^{125}I from the FSH molecule means that the exogenous FSH has been catabolized, the results of the gel-filtration data indicate that unabsorbed FSH is catabolized at a relatively slow rate. Results of a preliminary study in sheep showed that the blood levels of radioactivity at 24 hr after an i.m. injection of [^{125}I]FSH were only 8% of the levels at 320 min. This indicates that the FSH has been either absorbed and catabolized or that catabolism of the unabsorbed hormone occurred within 24 hr after injection.

It seems that the 40% loss of ^{125}I from the FSH molecule in the serum from blood samples taken 64 min or longer after i.v. administration of the labelled hormone would decrease the significance of disappearance rates, in terms of biological half-life, when they are calculated from values beyond 64 min. Kohler *et al.* (1968) reported no difference in metabolic clearance rates of human [^{125}I]FSH in women between a single injection and constant infusion of the labelled hormone. This does not mean that part of the estimated disappearance rate is based entirely on the loss of FSH; a part of it is based on the loss of free, radioactive iodine.

A slower disappearance rate of labelled FSH in the rats, which had been ovariectomized 21 days before treatment, might indicate that higher levels of endogenous FSH present in castrate rats (Gay, 1970) decrease the rate of catabolism of exogenous FSH. Lower blood levels of the labelled hormone in these rats as compared with those treated during oestrus, dioestrus or 2 days after ovariectomy, however, would not support this explanation.

There was a difference in the regression coefficient of the radioactive decay curve among the different species studied, but the shape of the curve for disappearance and uptake of [^{125}I]FSH was similar in all species. When all values within each species were expressed as a percentage of the highest counts/min, there was no difference in either disappearance or uptake rates. Thus, it is concluded that results of studies on uptake rates of exogenous FSH in the rat or rabbit would be similar to those in the ewe and cow.

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