Species Specificity of FSH and LH as Determined by Radioimmunoassay.* (32322)

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The recent development of sensitive and specific radioimmunoassays for human FSH (1) and LH (in preparation) has enabled us to study the question of immunological cross-reactivity of these hormones with those from other species. The finding of immunologic species specificity for both FSH and LH will be reported herein.

Materials and methods. Partially purified preparations of ovine, bovine, porcine, equine and canine pituitary gonadotropins were prepared by procedures published elsewhere (2-5). Pituitary extracts from the remaining species† (Tables I and II) were prepared by homogenizing the fresh frozen glands with cold 0.9% saline in a Waring Blendor for 3 minutes. The insoluble residue was separated by centrifugation at 4°C and discarded. The protein concentration of such extracts was determined by the Folin-phenol method(6). Pregnant mare serum gonadotropin (PMSG) was purchased from a commercial source.‡ LH activity was determined by the ovarian ascorbic acid depletion method of Karg(7) and Parlow(8) and FSH activity by the ovarian-weight gain method of Steelman and Pohley(9). The details of the bioassays were as previously described(10).

The radioimmunoassay technique for human FSH has been previously reported(1) A similarly sensitive and specific double-antibody radioimmunoassay for human LH has subsequently been developed. In this assay, highly purified human pituitary LH is used for radioiodination and standards, and an anti-LH antiserum prepared in guinea pigs against potent human pituitary LH fractions is employed for the first stage antiserum.

TABLE I. Cross-Reactivity Studies—FSH.

Source of fraction	Bio-FSH*	Immuno-FSH*	% Cross-reactivity
Human	95.2	90.8	95.3
Ovine	17.9	<.006	<.0034
Ovine-NIH-FSH-S2	.87	<.006	<.069
Bovine	1.3	<.004	<.031
Equine	3.5	<.013	<.037
Porcine	2.1	<.012	<.057
Canine	2.8	<.008	<.029
Rat†	.20	<.0011	<.55
Monkey, Rhesus	.11	<.011	<10.0
Monkey, Cynomolgus	<.09	<.0015	—
Mouse, Swiss Webster	>.27	<.0004	<.14
Rabbit, mixed breeds	>.17	<.0009	<.52
Hamster, golden Syrian	>.21	<.0005	<.23
Frog, *Rana pipiens*	<.32	<.0001	—
Turtle, *Chelydra serpentina*	<.08	<.0001	—
PMSG‡	6.9	<.012	<.017

* All values expressed as NIH-FSH-S1 U/mg (1 unit activity in 1 mg).

† Obtained from W. White, Abbott Laboratories, North Chicago, Ill.

‡ Expressed as U/ampoule.

Potency estimates for both the bioassays and immunoassays were expressed as units per mg dry weight or per mg of Folin-reacting protein. PMSG results were expressed as units per ampoule. NIH-FSH-S1 or NIH-LH-S1 were the reference preparations, and one unit was taken to be the activity in one mg of the appropriate standard.

Results. The results of the cross-reactivity of the pituitary fractions of the various species in the FSH assay are shown in Table I. It can be seen that there was little, if any, immunologic cross-reactivity of these fractions. Nothing definite can be stated regarding frog, turtle, and Cynomolgus monkey, for although there was no significant immunologic cross-reactivity, no biologic activity was demonstrable in the pituitary extracts from these species at the doses tested. However, the possibility of a significant degree of cross-

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† Obtained from Pel-Freez Biologicals, Rogers, Ark.

‡ Equinex-Ayerst Laboratories, New York.

TABLE II. Cross-Reactivity Studies—LH.

Source of fraction	Bio-LH*	Immuno-LH*	% Cross-reactivity
Human	2.80	2.56	91.4
Ovine	1.43	<.00004	<.0027
Ovine-NIH-LH-S5	.10	<.00004	<.040
Bovine	1.31	<.00006	<.0045
Equine	.20	<.00009	<.045
Porcine	.65	<.00004	<.0061
Rat†	.23	<.00005	<.022
Monkey, Rhesus	—	<.00001	—
Monkey, Cynomolgus	.025	<.00003	<.12
Mouse, Swiss Webster	.03	<.00001	<.033
Rabbit, mixed breeds	.03	<.00002	<.067
Hamster, golden Syrian	.095	<.000005	<.0053
Frog, *Rana pipiens*	—	<.00002	—
Turtle, *Chelydra serpentina*	.2	<.00001	<.005

* All values expressed as NIH-LH-S1 U/mg (1 unit activity in 1 mg).

† Obtained from W. White, Abbott Laboratories, North Chicago, Ill.

reactivity with the Rhesus monkey extract, albeit less than 10%, cannot be excluded.

The results of the cross-reactivity in the LH assay are shown in Table II. Also little, if any, cross-reactivity was observed. Bioassays were not performed on the extracts from Rhesus monkey and frog due to insufficient quantities of material.

Comments. Although immunological cross-reactivity of gonadotropins has been studied by various *in vitro* immunologic techniques and by biological neutralization studies, there has been only one previous report examining this problem through use of the sensitive and specific method of radioimmunoassay (11). In the present study, we have shown that, despite the biological effectiveness of a number of pituitary extracts from various species as assayed in the rat, no demonstrable immunologic cross-reactivity could be shown for either FSH or LH.

Due to inadequate quantities of certain pituitary extracts, no definitive conclusions can be reached for the frog and Rhesus monkey for both FSH and LH and the Cynomolgus monkey and turtle for FSH alone. At most, the cross reactivity for the Rhesus extract in the FSH system would be 10%. These data are in agreement with those of Neill *et al*(11) who observed no cross-reactivity with Rhesus or squirrel monkey in both LH and FSH radioimmunoassays. However, using a hemagglutination-inhibition technique a significant degree of cross-reactivity has been observed with crude simian LH and FSH(12) and with crude LH extracts from other species(13). The nature of these apparent discrepancies is unclear.

As discussed elsewhere(14,15), the results obtained from studies of cross-reactivity may depend on whether the criterion be antagonism of biological activity or the demonstration of *in vitro* cross-reactivity. For instance, antibodies to ovine(16) and bovine (in preparation) LH have been shown capable of neutralizing the biological activity of LH from certain species, whereas positive *in vitro* immunologic reactions against these hormones were not obtained. In addition, antagonism of endogenous hormone production using an anti-ovine LH serum has also been shown in both the rat(14,15) and in the rabbit(17). Failure to demonstrate *in vitro* antagonism may simply reflect solubility of the antigen-antibody complexes. However, it appears possible that the molecular sites for biologic effectiveness may differ from the sites of *in vitro* immunologic reactivity and antibodies may be directed against either or both of these sites. Thus it may be possible to antagonize biological effects of a hormone, without being able to demonstrate *in vitro* cross-reactivity.

Summary. Crude pituitary extracts and partially purified FSH and LH fractions from a variety of species have been shown to possess little, if any, cross-reactivity in hormone-specific radioimmunoassays for human FSH and LH. PMSG did not cross react in the FSH immunoassay.

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A Plate Method for Quantitation of Fibrinolytic Activity.* (32323)

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This report deals with a method for measuring fibrinolytic activity, based upon the principles used in the fibrin plate method of Astrup and Mullertz(1). Many investigators have modified the original Astrup and Mullertz procedure in order to overcome certain limitations of the method or to adapt it to a particular need. The method herein described facilitates the measurement of lysed areas, increases the accuracy of the measurement, minimizes the influence of a number of variables and requires a shorter incubation period. Although the techniques which this method employs are described in conjunction with the fibrin plate method they can also be applied to methods utilizing other substrates, such as plasma, and can be adapted to improve many of the modifications of the original Astrup and Mullertz procedure.

A new step introduced to the conventional fibrin plate method is the staining of the plate after incubation with the fibrinolytic agents(2). This innovation results in a striking contrast between stained unhydrolysed substrate and sharply demarcated transparent lysed areas (Fig. 1), thus facilitating the conventional manual measurement of the areas of lysis. More significantly, however, this procedure permits photometric quantitation of fibrinolytic activity. The latter is a rapid and more accurate technique than the conventional manual measurement of the lysed areas (*i.e.*, obtaining the product of two perpendicular diameters), particularly when the areas of lysis are irregular.

Materials and methods. Nine ml of 0.3% bovine fibrinogen (Armour) in phosphate buffer (pH 7.6) with an ionic strength of 0.15 are poured into a sterile flat bottomed petri dish (8.5 cm diameter) and clotted with 0.1 ml of thrombin solution (Topical Thrombin, Parke Davis, 100 NIH units per ml of 0.9% saline). Standardized movements of the dish are employed while thrombin is added to insure uniform mixing. Ten dishes placed on a level bench are used for each test. After clotting, the plates are allowed to stand for 15-30 minutes and then a 20-lambda drop of each test solution is placed on the surface of each plate. A plate can accommodate up to 8 drops without overlapping of the resulting areas of lysis. To minimize the influence of variables related to possible unevenness in the thickness of the fibrin plate or variations in its density, the dishes are rotated, each by a different arc, before application of the test solutions. Each sample drop is then placed on the surface of each plate on a site that has the same orientation in all dishes.

A practical method for placing the sample drops on the fibrin surface is to utilize a 1.0 ml capacity Hamilton syringe and a repeat-

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