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Competition for the *in Vitro* Binding of Radioiodinated Human Follicle-Stimulating Hormone in Reptilian, Avian, and Mammalian Gonads by Nonmammalian Gonadotropins

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Radioiodinated human FSH (^{125}I-hFSH) was used to study the specificity of the gonadotropin binding sites of various reptilian, avian, and mammalian gonadal tissues by examining competitive interactions with several nonmammalian gonadotropins. All preparations of nonmammalian gonadotropins showed some activity in these radioligand assays, but wide variations in activities were evident depending on the source of the tissue and source of the hormone. Several cases of marked species specificity in binding were apparent: Nonmammalian hormones were relatively inactive with porcine granulosa cells; frog and snake hormones were relatively inactive in turtle tissues; and frog hormones were essentially inactive in avian tissues. FSH–LH specificity of binding determined with hormones of nonmammalian origin differed significantly from that previously described with the aid of mammalian gonadotropins. In particular, when tested with some tissues, three preparations of LH, from the turkey, sea turtle, and frog, were in some cases more active in competing for ^{125}I-hFSH binding than preparations of FSH from the same species; this activity could not be readily accounted for by FSH contamination. These comparative data demonstrate the existence of considerable overlap in the binding characteristics of some species of FSH and LH; the observed differences probably reflect evolutionary changes in both gonadotropin binding sites and in the structure of the gonadotropins.

In a previous study (Licht and Midgley, 1976), we reported that radioiodinated human follicle-stimulating hormone (^{125}I-hFSH) showed high affinity binding to a wide array of reptilian and avian gonadal tissues; this binding was tissue specific and displayed many of the characteristics of mammalian FSH receptors. Radioligand assay with a variety of purified pituitary hormones from four mammalian species verified that this binding was specific for gonadotropins, but pronounced variations among species were evident in the specificity for the two types of gonadotropins. The testicular and ovarian tissues of all turtles and birds examined were like those of mammals in being highly specific for FSH; i.e., purified LH showed little competition for the binding of ^{125}I-hFSH. However, all preparations of mammalian LH showed appreciable activity in competition for ^{125}I-hFSH binding to the gonadal tissues of squamate reptiles (snakes and lizards), indicating that FSH and LH may share common binding sites in these reptiles.

The good indices of precision, high reproducibility, and sensitivity of the radioligand assays with reptiles and birds suggested that ^{125}I-hFSH binding might offer a valuable technique for quantifying the relative activities of gonadotropins in these nonmammalian species. However, nothing was known about the binding of homologous gonadotropins, and evaluation

of such a heterologous system required information on the relative activities and behavior of purified hormones from nonmammalian species. Comparative data are especially important for understanding FSH–LH specificity, since physiological studies have indicated that the responses of reptiles to mammalian FSH and LH do not always parallel those observed with the two homologous hormones (e.g., Crews and Licht, 1975; Licht and Crews, 1976; Tsui, 1976).

The present investigation extends the analysis of gonadotropin binding sites in reptilian and avian gonadal tissues by examining the relative abilities of various nonmammalian hormones to compete for the binding of ^{125}I-hFSH. In particular, radioligand assays were performed with ovarian and testicular tissues from representatives of two orders of reptiles and two orders of birds, using hormones derived from several amphibian, reptilian, and avian species. Parallel measurements were also made with a mammalian gonadal preparation, i.e., porcine granulosa cells, to examine further the problem of phylogenetic specificity in gonadotropin actions.

MATERIALS AND METHODS

Preparation of tissues and iodination of hFSH. The methods used for the iodination of hFSH, preparation of tissues, and procedures for competitive studies were the same as described previously (Licht and Midgley, 1976). The gonadal tissues examined here also included the same variety as used in our initial study with mammalian gonadotropins: for reptiles, Order Chelonia (snapping turtle, *Chelydra serpentina;* soft-shelled turtle, *Trionyx spiniferus;* painted turtle, *Chrysemys picta;* green sea turtle, *Chelonia mydas*) and Order Squamata (garter snake, *Thamnophis sirtalis;* anole lizard, *Anolis carolinensis*); for birds, Order Anseriformes (mallard duck, *Anas playrhynchos*) and Order Galliformes (chicken, *Gallus domesticus*). Although a relationship between *in vitro* binding to components in these tissues and a physiological response has not yet been established, for heuristic purposes, the tissue homogenates are referred to as "receptor" preparations.

Radioligand assays were performed by incubating 50 μl of gonadal homogenate (1.5–3.0 mg wet weight tissue pellet), 50 μl of radioligand (50,000–75,000 cpm), and 50 μl of buffer or hormone solution in 12 × 75-mm polypropylene tubes. Reaction mixtures were incubated at 30° for 3 hr. The relative activities of hormones were computed in terms of the ability of the hormones to inhibit the binding of ^{125}I-hFSH by comparison with a single reference preparation of highly purified human FSH (AFP-574C). Parallel line statistics (Finney, 1964) were used in conjunction with percentage logit vs log dose–response curves; no weighting procedures were employed, but analyses were restricted to the central (20–80%) region of the inhibition curve. As in earlier tests with mammalian hormones, indices of precision (lambda) ranged from 0.05 to 0.18; repetitive estimates for the same solution rarely varied by as much as twofold. No consistent differences in dose–response slopes were observed among different species of hormones or between FSH and LH.

Hormones. Tests were performed with purified preparations of FSH and LH from several reptiles, the turtles (*Chelydra serpentina* and *Chelonia mydas*) and the alligator (*Alligator mississipiensis);* birds (duck, chicken, and turkey); and an amphibian (bullfrog, *Rana catesbeiana*). In addition, an enriched gonadotropin fraction from duck and several crude pituitary extracts from turtles and snakes were examined. The procedures for purification and bioassay of these nonmammalian hormones are detailed in Licht and Papkoff, 1974a,b; Licht *et al.,* 1976a; Farmer *et al.,* 1975; Papkoff *et al.,* 1976a,b). The FSH potency was initially estimated from a lizard testis maintenance bioassay with NIH-FSH-S10 as a reference (Table 1). Preparations of differing purity, as well as crude pituitary extracts, were tested from each species. There was generally good agreement between relative activities estimated by bioassay and radioligand assay for preparations from one species, except for the frog hormones (see below). For the present discussion attention is focused on the most highly purified preparations available for each species.

To facilitate comparison between data from bioassay and radioligand assay, relative activities were computed in terms of the same hFSH reference (Table 1) by assuming that this preparation would show approximately the same relative activity in the lizard as in Steelman–Pohley assay (about 56 × NIH-FSH-S1). Previous studies with purified ovine FSH provide a basis for this assumption (Licht and Pearson, 1969). Each hormonal preparation was assayed at least twice, in some cases up to six times, with each species of receptor.

RESULTS

Reptilian hormones. The relative abilities of reptilian gonadotropins to compete for the binding of ^{125}I-hFSH to various receptor

TABLE 1
RELATIVE POTENCIES OF REPTILIAN, AVIAN, AND AMPHIBIAN GONADOTROPINS OBTAINED BY BIOASSAY IN *Anolis* LIZARD

Preparation	Identification[a]	Relative potency[b]		LH/FSH potency ratio[c]
		X NIH-FSH-S1	X hFSH (AFP-574C)	
Snapping turtle (*Chelydra serpentina*)				
FSH	T-48-C	30	0.54	0.004
LH	T-28B,37-C	0.12	0.002	
Green sea turtle (*Chelonia mydas*)				
FSH	TC-47CRC	3.3	0.06	0.3
LH	TC-48-B	1.0[d]	0.018	
Alligator (*Alligator mississipiensis*)				
FSH	W-208-D	2.5	0.044	0.4
LH	W-144-C	1.0[d]	0.018	
Chicken FSH	W-124-B	4.0	0.07	
Turkey				
FSH	W-127-BSD	4	0.07	0.075
LH	W-28-BG	0.3	0.005	
Duck FSH/LH	W-246-B	3.0	0.05	—
Bullfrog (*Rana catesbeiana*)				
FSH	C-61BH	2.5	0.044	
FSH	C-164-B	7.5	0.133	0.016
LH	C-53-C	0.12	0.002	

[a] Details on preparation of hormones are reported in: *Chelydra* (Papkoff *et al.*, 1976a); *Chelonia* and *Alligator* (Licht *et al.*, 1976b); turkey (Farmer *et al.*, 1975); chicken and duck (Farmer, Papkoff, and Licht, unpublished); *Rana* (Licht and Papkoff, 1974b; Papkoff *et al.*, 1976b).

[b] All preparations were assayed against NIH-FSH-S10 (=1 × NIH-FSH-S1) as a reference. Potencies were converted in terms of the human FSH reference (AFP-574C) assuming that the latter would show the same relative potency in the lizard assay as in the Steelman–Pohley assay (=56 × NIH-FSH-S1).

[c] Ratio of potency between the LH and the most potent FSH from each species.

[d] These estimates are only approximations since dose–response curves of LH were not parallel to those for FSH.

preparations are presented in Tables 2 and 3. All three species of reptilian FSH were able to inhibit the binding of ^{125}I-hFSH to all receptor preparations, but pronounced differences in activity were evident among the various species of hormone and receptor. Porcine granulosa cells were the least able to bind reptilian hormones; potencies of reptilian FSH in the mammalian receptor assay were from 2.4- to 33-fold less than those observed in systems using the same labeled mammalian hormone with non-mammalian receptors (Tables 2 and 3). Reptilian and avian receptors did not differ consistently in their ability to bind reptilian hormones.

The FSH from the turtle *Chelydra* was consistently the most active of the three preparations of reptilian FSH tested; the activities of *Alligator* and *Chelonia* FSH were approximately the same in any given system. However, each preparation of FSH showed a variation in activity of about four- to sixfold among the different reptilian receptors. Hormones were not more active when tested in the homologous receptor than in heterologous ones (e.g., in the case of *Chelonia* and *Chelydra*, Table 1).

Preparations of LH from the three reptilian species varied widely in their ability to compete for the binding of ^{125}I-hFSH. Of particular importance is the comparison of their activities relative to the FSH from the same species (Fig. 1). These LH/FSH potency ratios demonstrate that *Chelydra* LH exhibited relatively low binding activity in

TABLE 2
RELATIVE ACTIVITIES OF REPTILIAN PITUITARY GONADOTROPINS OBTAINED BY RADIOLIGAND ASSAY UTILIZING THE BINDING OF ^{125}I-hFSH TO TURTLE GONADAL TISSUES

Hormone and source	Identification	Tissue preparation[a]				
		Chrysemys testis	*Chelydra* testis	*Trionyx* ovary	*Chelonia* testis	*Chelonia* ovary
Snapping turtle (*Chelydra serpentina*)						
FSH	T-48-C	0.55	0.25	0.23	0.29	—
LH	T-28B,37C	0.008	0.009	0.02	<0.005	
Sea turtle (*Chelonia mydas*)						
FSH	TC-47 CRC	0.09	0.20	0.045	0.17	0.09
LH	TC-48-B	0.10	0.034	0.11	0.02	0.03
Alligator (*Alligator mississipiensis*)						
FSH	W-192-D	0.16	—	0.08	0.14	—
LH	W-144-C	0.015	—	0.027	0.016	—

[a] All activities are expressed in terms of a human FSH reference standard (AFP-574C).

TABLE 3
RELATIVE ACTIVITIES OF REPTILIAN PITUITARY GONADOTROPINS OBTAINED BY RADIOLIGAND ASSAY UTILIZING THE BINDING OF ^{125}I-hFSH TO SQUAMATE AND AVIAN TESTES AND PORCINE GRANULOSA CELLS

Hormone	Identification	Testis preparation				Porcine granulosa
		Snake	Lizard	Chicken	Duck	
Snapping turtle (*Chelydra*)						
FSH	T-48-C	1.8	0.82	0.42	—	0.09
LH	T-28B,37C	0.026	0.068	0.015	—	0.006
Sea turtle (*Chelonia*)						
FSH	TC-47-CRC	0.15	0.11	0.16	0.05	0.006
LH	TC-48-B	0.15	0.36	0.16	0.025	0.025
Alligator (*Alligator*)						
FSH	W-192-D	0.37	0.27	0.09	0.09	0.033
LH	W-144-C	0.0065	0.027	0.015	0.015	0.023

tests with all receptors (i.e., it was less than 10% as active as *Chelydra* FSH). In contrast, LH from the second turtle (*Chelonia*) was, in most cases, either equipotent or even severalfold more active than the corresponding FSH. This LH had the lowest relative activity when tested with homologous, *Chelonia*, receptor preparation. The relative activity of *Alligator* LH was low like that of the *Chelydra* hormone except in tests with the mammalian receptor preparation (Table 3, Fig. 1).

Tests with crude pituitary homogenates of turtle, snake, and lizard pituitaries revealed marked species specificity among reptilian receptors. The activities in these homogenates were approximately equal when tested in radioligand assay with the snake receptor preparation but snake and lizard pituitary preparations were essentially inactive when tested with turtle receptors. For example, pituitaries from diverse snakes (*Bungarus*, *Ptyas*, and *Thamnophis*) and the lizard (*Anolis*) had poten-

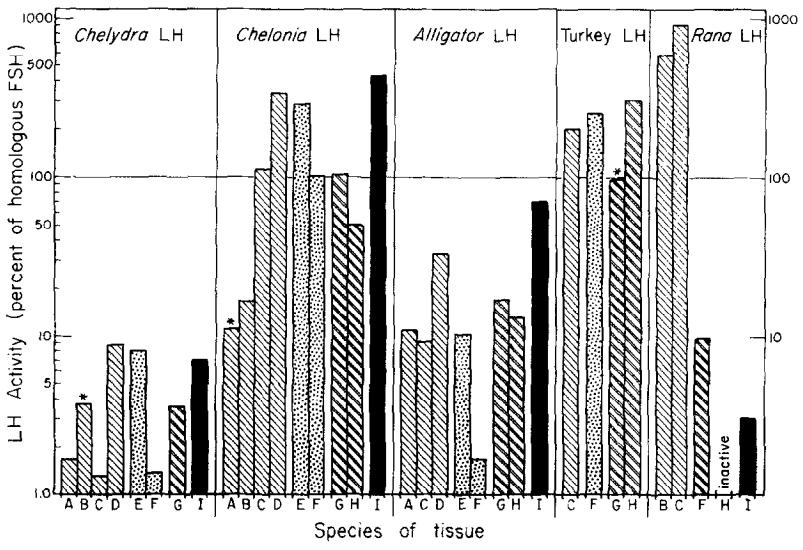

FIG. 1. Relative activities of LH from five nonmammalian species obtained by radioligand assay employing ^{125}I-hFSH binding to gonadal tissues from nine species (A–I). The activity of each preparation of LH is compared to that of the FSH from the same source; thus, an activity of 100% indicates that LH was equipotent to FSH in competing for the binding of ^{125}I-hFSH. The different species from which tissues were tested are: Order Chelonia (A, *Chelonia;* b, *Chelydra;* C, *Chrysemys;* D, *Trionyx*); Order Squamata (E, *Anolis;* F, *Thamnophis*); Aves (G, chicken; H, duck); Mammalia (I, porcine). An asterisk indicates those cases in which the species of tissue was homologous to the species of hormone being tested (same species or genus). Values are computed from data in Tables 2–5.

cies of 20% or more of the hFSH standard when tested with snake receptors, but their potencies were *less than* 0.3% of hFSH in tests with turtle receptors.

Avian hormones. Relative activities of the hormones from the three birds (duck, chicken, and turkey) were highly variable among receptors, and results for avian LH paralleled those for *Chelonia* hormones (Table 4). Although the relative activities of the three preparations of avian-FSH were comparable in *Anolis* bioassay, they were markedly divergent in radioligand assay. In general, they were most active in the snake receptor system and least active in turtle and duck; the ranking of activity also varied for each receptor. The activity of the one purified avian LH (turkey) was either the same or greater than that of the homologous FSH in all cases (Fig. 1).

TABLE 4

RELATIVE ACTIVITIES OF AVIAN GONADOTROPINS OBTAINED BY RADIOLIGAND ASSAY USING REPTILIAN AND AVIAN TESTES WITH ^{125}I-hFSH[a]

		Tissue (testis)			
Hormone	Identification	Snake	Turtle	Chicken	Duck
Chicken FSH	W-124-B	0.06	0.006	0.02	0.01
Turkey FSH	W-127BSD	0.02	0.003	0.008	0.0013
Turkey LH	W-28BG	0.05	0.006	0.007	0.0034
Duck FSH/LH	W-246B	0.19	0.030	0.0065	0.013

[a] *Activities are computed with reference to highly purified human FSH (AFP-574C).*

TABLE 5
RELATIVE ACTIVITIES OF BULLFROG GONADOTROPINS OBTAINED BY RADIOLIGAND ASSAY USING REPTILIAN AND AVIAN TESTICULAR HOMOGENATES AND PORCINE GRANULOSA CELLS

Preparation	Identification	Tissue				
		Chelydra	*Chrysemys*	Snake	Duck	Porcine granulosa
FSH	C-61-BH	0.001[a]	0.001	0.08	0.0010	0.008
	C-164-B	—	0.001	0.041	0.0001	—
LH	C-153-C	0.006	0.009	0.008	0.0010	0.0003

[a] Activities in radioligand assay based on comparison to an hFSH (AFP-574C) standard.

Amphibian hormones. The behaviors of the bullfrog FSH and LH were also variable among receptors and their activities were inconsistent with bioassay (Table 5). Even though the *Rana* FSH was one of the most potent nonmammalian preparations in bioassay (Table 1), it tended to be relatively inactive in all binding tests (Table 5). LH/FSH activity ratios also varied widely (Fig. 1). Although both frog hormones possessed extremely low inhibitory activity with porcine granulosa cells, the FSH was considerably more potent than LH. Likewise, *Rana* LH was only 10% as active as FSH in tests with snake tissues. However, with the two turtle tissues, *Rana* LH was almost 10 times *more* active than the FSH (Fig. 1). These differences in LH/FSH ratio were due primarily to variations in activity of FSH, since LH possessed equivalent activity when tested with tissue preparations from snake and turtle.

DISCUSSION

These studies confirm that the binding of ^{125}I-hFSH to reptilian and avian tissues is associated with the sites involved in the binding of nonmammalian gonadotropins and that a radioligand system employing a mammalian hormone can be used to analyze nonmammalian hormones quantitatively. However, several problems exist for the interpretation of data obtained with these heterologous binding systems.

Species specificity in the "recognition" of different hormones by the ^{125}I-hFSH binding sites may present an important problem in heterologous assay systems. The maximal activities of the nonmammalian hormones, as judged by their ability to inhibit the binding of ^{125}I-hFSH, are slightly less than those of several highly purified preparations of mammalian FSH (cf. Licht and Midgley, 1976). It is not clear whether these results reflect slight differences in the purities or in the affinities of the different species of hormone. In contrast, nonmammalian hormones show uniformly low activities when tested with a mammalian FSH receptor preparation. This latter case of species specificity in the mammalian receptor is consistent with the relative inactivity of purified nonmammalian hormones in mammalian bioassays (e.g., Licht *et al.*, 1976b; Papkoff *et al.*, 1976b). Channing similarly reported (in Reichert *et al.*, 1973) that turtle gonadotropin was ineffective in competing for the binding of radioiodinated human chorionic gonadotropin to mammalian granulosa cells. Reichert *et al.* (1973) reported that, while avian (chicken) gonadotropin of unstated potency competed for the binding of ^{125}I-hLH to rat testes, it gave a significantly lower competitive inhibition slope than did mammalian-LH.

Nonmammalian hormones do not bind equally well to all nonmammalian tissue preparations. For example, bird, snake, and frog hormones were relatively inactive when tested by radioligand assay with turtle tissues, and frog hormones were very inactive when tested with bird tissues (Ta-

ble 5). Similar cases of specificity have been observed in physiological experiments: In studies of *in vitro* testicular steroidogenesis, snake and frog hormones were potent when tested in snakes but inactive with bird tissues; snake hormones were also relatively inactive when tested in turtles (Tsui, 1976). These results suggest that the structural characteristics of the FSH molecule required for binding and the nature of the binding sites for FSH have each undergone evolutionary change.

In addition to species specificity at the levels of the hormone and the receptor, pronounced variations in the ability to discriminate FSH from LH were also observed for the various tissues examined. In previous studies with mammalian gonadotropins, the binding of ^{125}I-hFSH to tissues of birds and turtles appeared to be highly specific for FSH (Licht and Midgley, 1976). Squamate tissues showed less specificity since some preparations of LH, especially bovine, were able to compete significantly for the binding of ^{125}I-hFSH; however, FSH was always more potent than LH. These results contrast markedly with those obtained in this study with LH from nonmammalian origin, wherein, depending on the species of tissue and on the species of hormone, an LH may be even more potent than the FSH from the same species.

The *Chelonia* and *Rana* hormones provide a particularly striking example of overlap in LH and FSH activity. When tested in *Chelonia* and *Chelydra*, the *Chelonia* LH was relatively inactive; but with all other receptor preparations, it was equipotent or several times more potent than *Chelonia* FSH (Fig. 1). This phenomenon is clearly not a characteristic of LH from all turtles or reptiles (Fig. 1). A similar situation was evident for *Rana* LH. The turkey LH was relatively active with all species of receptor (Fig. 1). These variations make it unlikely that the instances of high activity in preparations of LH can be attributed to FSH contamination. Bioassay data (Table 1) and chemical information on these preparations (Farmer *et al.*, 1975; Licht *et al.*, 1976a; Papkoff *et al.*, 1976a,b) also argue against a high contamination by FSH. Radioimmunological studies in progress (Daniels and Licht, unpublished) also indicate that the cross-contamination between *Rana* FSH and LH is less than 0.2%.

The apparent overlap in binding activity among preparations of FSH and LH raises the possibility that the human FSH used as the ligand is binding to a variety of sites normally associated with two different types of gonadotropin molecule in the nonmammalian gonad; i.e., the hFSH shares the binding properties of a nonmammalian FSH and LH. However, if this were true, biphasic, nonparallel inhibition curves should have been obtained. In any case, it is apparent that considerable evolutionary divergence has occurred in the structure of gonadotropin molecules and their binding sites. It appears that with certain tissues, LH of one species may resemble the FSH of another species; in fact, this similarity may be greater than that between preparations of FSH from the same two species. It is also important to recognize that such divergence in hormones or binding sites may be greater among members of the same order (e.g., *Chelonia* vs *Chelydra*) than among members of different classes or orders. The difference between the two species of turtles is not unexpected in view of the antiquity of the two families that they represent (Romer, 1966).

These comparative data indicate that a radioligand assay employing heterologous reagents can be used to study the binding of nonmammalian gonadotropins to the tissues of nonmammalian species. However, conclusions regarding the properties of the binding sites in such heterologous systems must be made with considerable caution. In particular, definition of FSH–LH specificity in terms of mammalian hormones may be misleading, and extrapolation of such findings from one species to another,

even among taxonomically related groups, may be inaccurate. Although the FSH and LH molecules of nonmammalian species are sufficiently different from one another to be separated by various chemical techniques (e.g., ion-exchange chromatography) and to be recognizable by certain chemical characteristics (e.g., carbohydrate composition and amino acid composition (Farmer *et al.*, 1975; Licht *et al.*, 1976a; Papkoff *et al.*, 1976a), they may show considerable overlap in those properties related to binding activity. Further clarification of the evolution of gonadotropin binding specificity requires additional study using nonmammalian hormones, including LH, as ligands.

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REFERENCES

Crews, D., and Licht, P. (1975). Stimulation of *in vitro* steroid production in turtle ovarian tissue by reptilian, amphibian and mammalian gonadotropins. *Gen. Comp. Endocrinol.* **27,** 71–83.

Farmer, S. W., Papkoff, H., and Licht, P. (1975). purification of turkey gonadotropins. *Biol. Reprod.* **12,** 415–422.

Finney, D. J. (1964). "Statistical Methods in Biological Assays," 2nd ed. Griffin, London.

Licht, P., and Crews, D. (1976). Gonadotropic stimulation of *in vitro* progesterone production in reptilian and amphibian ovaries. *Gen. Comp. Endocrinol.* **29,** 141–151.

Licht, P., and Midgley, A. R., Jr. (1976). *In vitro* binding of radioiodinated human follicle-stimulating hormone to reptilian and avian gonads: Radioligand studies with mammalian hormones. *Biol. Reprod.*, (in press).

Licht, P., and Papkoff, H. (1974a). Separation of two distinct gonadotropins in the pituitary gland of the snapping turtle (*Chelydra serpentina*). *Gen. Comp. Endocrinol.* **22,** 218–237.

Licht, P., and Papkoff, H. (1974b). Separation of two distinct gonadotropins from the pituitary gland of the bullfrog *Rana catesbeiana*. *Endocrinology* **94,** 477–483.

Licht, P., and Pearson, A. K. (1969). Effects of mammalian gonadotropin (FSH and LH) on the testes of the lizard *Anolis carolinensis*. *Gen. Comp. Endocrinol.* **13,** 367–381.

Licht, P., Farmer, S. W., and Papkoff, H. (1976a). Further studies on the chemical nature of reptilian pituitary gonadotropins: FSH and LH in the American alligator and green sea turtle. *Biol. Reprod.* **14,** 222–232.

Licht, P., Muller, C., and Tsui, H. W. (1976b). Effects of mammalian and nonmammalian gonadotropins on androgen production by minced rabbit testis. *Biol. Reprod.* **14,** 194–201.

Papkoff, H., Farmer, S. W., and Licht, P. (1976a). Isolation and characterization of follicle stimulating hormone and luteinizing hormone and its subunits from snapping turtle (*Chelydra serpentina*) pituitaries. *Endocrinology* **98,** 767–777.

Papkoff, H., Farmer, S. W., and Licht, P. (1976b). Isolation and characterization of luteinizing hormone from amphibian (*Rana catesbeiana*) pituitaries. *Life Sci.* **18,** 245–250.

Reichert, L. E., Jr., Leidenberger, F., and Trowbridge, C. G. (1973). Studies on luteinizing hormone and its subunits: development and application of a radioligand receptor assay and properties of the hormone-receptor interaction. *Recent Progr. Horm. Res.* **29,** 497–532.

Romer, A. (1966). "Vertebrate Paleontology," 3rd ed. University of Chicago Press, Chicago.

Tsui, H. W. (1976). "Gonadotropin Control of Androgen Production in Reptilian and Avian Testes." Ph.D. Thesis, University of Calif., Berkeley, Calif.