RELEASE OF GONADOTROPIN FROM THE ANTERIOR PITUITARY GLAND OF FOWL BY LH-RH[1] AS MEASURED BY THE ^{32}P UPTAKE OF THE TESTES.

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Summary: Single comb White Leghorn cockerels received LH-RH in varying dosages at different time periods. The uptake of ^{32}P by the testes was the end-point of the assay. Dosages as low as 0.06 µg were effective but at least 30 minutes were required for a significant response. Differences in ^{32}P uptakes were observed between 2-day and 8-day-old birds. The younger chicks had greater percentage increases over their controls than did the older chicks and the latter reached their peak response 40 minutes later. Assay of anterior pituitary glands demonstrated that 90 minutes after LH-RH treatment there was less gonadotropin present than in control glands but 150 minutes after injection anterior pituitary gland content was greater than that of the control glands.

Excellent responses have been elicited in the fowl by the administration of LH-RH and of hypothalamic extracts. These responses have ranged from variations in the luteinizing hormone (LH) levels in plasma and LH release from dispersed anterior pituitary cells *in vitro*, to the induction of ovulation in pullets (1,2,3,4,5). The experiments reported here, however, were designed to ascertain if significant increases in the ^{32}P uptake of the testes of cockerels would occur following subcutaneous administration of LH-RH. This technique does not differentiate between the release of LH only or the release of both LH and FSH. We have, therefore, referred in this paper to the release of "gonadotropin" rather than of LH.

Materials and Methods: Single-comb White Leghorn cockerels at two, eight, and nine days post-hatching were injected subcutaneously at various time intervals with different dosages of LH-RH. The ^{32}P uptake by the testes was determined and the counts-per-minute (Cpm) were divided by the square root of testes weights in milligrams and multiplied by the square root of the body weight in grams (6). These data were subjected to logarithmic transformation

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(7): Antilog $[L_{10}(\sqrt{Cpm_{Testes}} \times \sqrt{V_{Body}})]$ which reduced the variation within each group. Since logarithms were employed, the use of mean ± standard deviation (SD) is inappropriate, and, therefore, the 5% confidence limits for means were determined and are presented in the tables. Analysis of variance (Anova) was employed for comparisons. A control group was matched with each injected group at each time interval and percentages also were determined by randomized comparisons between individual responses in the respective control and treated groups. These were expressed as: Antilog $[L_{10}(\frac{\text{Treated Cpm}}{\text{Control Cpm}} \times 100)]$. Variation was slightly increased by this procedure as is demonstrated by the higher CV and S^2 values.

Results and Discussion: Administration of LH-RH at 0.06 µg, 0.12 µg, and 0.24 µg 90 minutes before autopsy produced definite increases in ^{32}P uptake by the testes indicating that the LH-RH had stimulated the AP to release gonadotropin. The 0.03 µg dosage, however, did not affect the counts significantly (Table 1). The F-value for the comparison of all LH-RH groups with all the controls was 26.65, $P<2.76^{-06}$. The control groups differed in uptake

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Table 1

Uptake of ^{32}P by the testes of 2-day-old cockerels given LH-RH 90 minutes before autopsy. Treated and control animals received 1.0 µCi ^{32}P one hour before autopsy, f=9, N=72.

^{32}P Testis Uptake

(A) LH-RH

	0.03 µg	0.06 µg	0.12 µg	0.24 µg
$\bar{x}$ [1]	223.12	305.56	304.57	320.08
Range [2]	198.40 to 250.95	236.10 to 395.45	270.51 to 342.95	287.38 to 357.49
CV [3]	2.83%	5.86%	2.70%	2.43%
		Controls		
$\bar{x}$	223.68	249.94	211.87	205.90
Range	203.20 to 246.21	195.67 to 319.26	192.03 to 233.76	182.12 to 232.80
CV	2.31%	5.77%	2.39%	3.00%

			MSO	F	P
ANOVA:	Treatments	7	0.558	7.00	3.81^{-06}
	LH-RH	1	0.2132	26.65	2.76^{-06}
	Error	63	0.5040	S^2=0.0080	

(B) Percent Increases - Treated vs. Controls

	0.03 µg	0.06 µg	0.12 µg	0.24 µg
$\bar{x}$	-3.56 [4] (2.40) [5]	38.44 (23.65)	41.45 (47.47)	75.04 (76.98)
Range	-18.05 to 13.48	0.54 to 90.62	22.9 to 63.34	51.23 to 102.23
CV	4.63%	8.44%	3.78%	3.68%

			MSQ	F	P
ANOVA:	Treatments	3	0.1038	7.63	5.52^{-04}
	Error	32	0.4352	S^2=0.0136	

1. Mean 2. 5% Confidence Limits 3. $\frac{SD}{\bar{x}}$ X 100 4. Net increase 5. Estimated values from Equation $\bar{Y} = 1.8848 + 0.2631X$

of ^{32}P which produced an additional variable in the analysis and evaluation of the data. This variation in testicular responses at different times of the day has been observed by others (8) and it was decided, therefore, to calculate the differences in responses as percentages between control and treated animals.

The percentage values adhered to the pattern shown by the actual ^{32}P uptakes: the 0.03 µg ranges overlapped those of the control but the increase for each of the three higher doses exceeded its respective control. The equation of the line gave good agreement for actual and estimated values except at the 0.06 µg dosage. This group, however, had the greatest variation with a CV of 5.86% for actual values and of 8.44% for percentages.

It was decided to administer 2.0 µg LH-RH to all cockerels in subsequent experiments to assure that the LH-RH level would be adequate for the release of gonadotropin from the anterior pituitary. Comparisons were made of ^{32}P uptakes using 2-day-old and 8-day-old chicks following the administration of LH-RH at various time intervals before autopsy. The percentage increases over the controls had several important features (Table 2). First ^{32}P uptake

Table 2

Uptake of ^{32}P by the testes of 2- and 8-day-old cockerels at different times following injection of 2.0 µg LH-RH. Each animal received 1.0 µCi ^{32}P one-hour before autopsy, f=9, N=144(156*).

Percentage Increases

Minutes[1]	$\bar{x}$ [2]	2 Day ♂ Range[3]	CV[4]	Minutes	$\bar{x}$	8 Day ♂ Range	CV
15	-5.98	-17.10 to 6.64	3.60%	15	12.82	-5.19 to 32.85	4.50%
30	25.72	9.30 to 44.62	3.77%	30	17.73	6.53 to 30.11	2.73%
60	42.33	19.76 to 69.17	4.53%	60	36.92	15.21 to 62.75	4.57%
90	76.19	42.62 to 117.66	5.32%	90	55.80	33.44 to 81.91	3.99%
110	156.49	110.85 to 212.00	4.59%	110	63.93	31.45 to 104.43	5.63%
130	120.37	79.78 to 170.13	4.91%	130	69.12	46.01 to 95.86	3.72%
150	109.80	69.91 to 159.05	5.13%	150	93.13	71.37 to 117.64	2.95%
				180*	43.41	21.84 to 68.80	5.92%
				240*	27.83	12.70 to 44.99	4.69%

1. Minutes after injection 2. Net increase 3. 5% Confidence Limits
4. $\frac{SD}{\bar{x}} \times 100$ * 15 animals per group

was not significantly increased when hormone was injected only 15 minutes before autopsy. This agrees with the observation that gonadotropin must be available for a minimum of 15 or 20 minutes in order to be effective for testicular ^{32}P uptake in cockerels (9). Second, the increases at 30 minutes were significantly different from the controls in both age groups, however, the percentage increases in the 2-day-old males was consistantly greater than in the 8-day-old birds at all time intervals. Third, the maximum increase in ^{32}P uptake occurred at 110 minutes in the younger animals but the maximum was not reached until 150 minutes in the older chicks. Fourth, declines in responses were observed in each age group after the maximum was reached.

The differences between the ^{32}P uptakes of 2-day and 8-day-old chicks involve several factors. If only the counts per-minute per-milligram of testis are claculated for the control animals, the counts were 15.2% higher in the 8-day-old birds than in the 2-day-old birds. The percentage increases, therefore, were lower in the eight-day animals partially as a result of relatively higher control divisors. A more subtle factor also is involved which is a reflection of the method of data transformation. Both body weights and testes weights increase rapidly but not in the same proportion between the second and eight days post-hatching. The ratio of the square root of body weight to the square root of testis weight in the younger birds, was 22.3% greater than in the older chicks. The data for the 2-day-old birds, therefore, were elevated by two variables: greater relative body weight and lower control ^{32}P uptakes. Since the counts-per-minute per-milligram of control testis tissue is greater, however, in the 8-day-old chicks, this suggests that a greater secretion of gonadotropin by the anterior pituitary gland may be present in the older cockerels. The release of gonadotropin following the administration of LH-RH, however, obviously does not increase the testicular uptake of ^{32}P of the 8-day-old chicks synergistically. Actually, since the responses were relatively lower with respect to those of the control testes there may be less release of gonadotropin by LH-RH injec-

tion or the testes may not be able to respond maximally to the gonadotropin. Reference to Table 1 and a comparison of the 0.24 µg dosage with the 2.0 µg dosage in Table 2 indicates that when the dose threshold had been reached either the LH in the anterior pituitary was completely discharged or that testicular response was limited.

It was important to determine if the assay could detect changes in the gonadotropin content of the anterior pituitary glands of chicks which had received LH-RH at different time periods (Table 3). This experiment varied slightly from the preceding ones inasmuch as 9-day-old birds were used and the ^{32}P was administered 70 minutes before autopsy to the donor animals (Series A). The means for the ^{32}P uptakes of the donor testes were greater than those observed in 8-day-old chicks for the same time periods (Table 2). The 5% confidence limits, however, overlapped in both. Anterior pituitary glands from the three donor groups and uninjected controls were administered to 3-day-old assay animals at only one dosage level, namely, 1.0 mg equivalent of fresh gland (Series B). The dosage was limited by the small quantity of anterior pituitary glands available.

Table 3

A. Nine-day-old cockerels were given 2.0 µg LH-RH at 30, 90, and 150 minutes before autopsy. 1.0 µCi ^{32}P was administered 70 minutes before autopsy, f=18, N=54. These animals were the donors of anterior pituitary glands for the assay of gonadotropin in Series B.

B. Three-day-old cockerels each received the equivalent of 1.0 mg of fresh anterior pituitary gland 4 hours before autopsy and 1.0 µCi ^{32}P 1 hour before autopsy, f=13, N=39.

A. 9-Day Old Donor Animals				B. 3-Day Old Assay Animals		
Minutes[1]	$\bar{x}$	Range[3]	CV[4]	$\bar{x}$	Range	CV
30	57.91	40.32 to 77.70	4.69%	-2.12	-14.22 to +11.70	3.25%
90	61.03	45.41 to 78.34	4.04%	-16.25	-25.43 to -5.93	4.34%
150	121.92	100.50 to 145.62	3.78%	+21.98	+5.29 to +41.32	5.07%

1. Minutes after injection 2. Net increase 3. 5% Confidence limits,
4. $\frac{SD}{\bar{x}} \times 100$

The anterior pituitary glands from those birds which were subjected to LH-RH for only 30 minutes produced a mean ^{32}P uptake which was within the normal range of the 5% confidence limits. The ^{32}P uptakes in the testes of assay chicks injected with anterior pituitary glands from 90-minute and 150-minute donors, on the other hand, deviated from the control. The uptake in the former was - 16.25% below the normal and the confidence limits were negative. The data for the 150-minute donor group, however, showed a marked increase in ^{32}P uptake of + 21.98% and the confidence limits did not overlap those of the 90-minute group. The F-value for the differences between the assay groups which received glands from the 90-minute and 150-minute donors had a probability of <0.0004. It can be concluded that real differences exist in the gonadotropin content of the anterior pituitary glands of animals which received LH-RH 90 minutes before autopsy and the glands of those injected with LH-RH 150 minutes before autopsy. Perhaps the higher level at 150-minutes indicates new gonadotropin synthesis.

It has been demonstrated that the ^{32}P uptake system in chicks provides an easy, rapid, inexpensive, and reliable assay that can be used to compare the relative effects of LH-RH and its analogues on gonadotropin release from the anterior pituitary gland.

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