LITHOSPERM[1] INHIBITION OF ANTERIOR PITUITARY HORMONES

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SUMMARY - A cold-water extract of Lithospermum ruderale was injected into White Leghorn chicks for nine days. Body weights decreased slightly but testes and thyroid weights were markedly depressed. Gonadotropin content of anterior pituitaries of donors given 5.0 mg daily of lithosperm was 300% greater, and in the 10.0 mg donors 2670% greater than control. Content in 20.0 mg donors, however, was only 23.8% of the control. Thyrotropin in the 5.0 mg donors was equivalent to the control but in the 10.0 mg donors, was 250% greater. The 20.0 mg donors, however, had only 51.9% as much thyrotropin as the control. It is suggested that, depending upon the amount, lithosperm can inhibit both the release and synthesis of gonadotropins and thyrotropin.

Extracts from various species of the plant genus Lithospermum have been shown to inhibit a number of protein hormones both in vivo and in vitro. The biological activity of TSH, FSH or LH, for example, can be decreased by in vitro incubation with lithosperm or in vivo when these hormones and lithosperm are administered separately at different injection sites (1). Lithospermic acid and a series of polyphenols have been isolated from plant roots and the relationship of these fractions to hormonal inhibition has been established (2,3,4,5), but the mechanism(s) of the anti-hormonal properties of lithosperm are unknown. There are several major possibilities and more than one could be involved in any given physiological or biochemical situation: a, inhibition might result from blocking the synthesis or release of a hormone; b, lithosperm might complex with a circulating hormone to inactivate it (6); or c, lithosperm might compete with a hormone for the receptor sites of target-cells. This paper is a report of an experiment designed to study the first

1. The term lithosperm is used as a generic designation for the hormone inhibiting principle, or principles, in the genus Lithospermum.

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alternative, that is, to determine if lithosperm will inhibit the synthesis and/or release of anterior pituitary gonadotropins and thyrotropin.

Materials and Methods. Single-comb White Leghorn cockerels were the experimental animals. One group of 37-day-old birds was injected daily with water for nine days and three groups were given lithosperm during the same period. All animals were killed on day 46 and the weights of the body, comb, testes, thyroids, and anterior pituitary glands were determined for each animal. Anterior pituitary glands were stored in acetone, then air-dryed, ground, suspended in distilled water, and assayed for gonadotropin and thyrotropin content. Dosages were based on wet-weights of the glands. The assay procedure utilized the uptake of carrier-free ^{32}P by the testes and thyroids with the anterior pituitary suspension being administered five hours before and 1.0 μ Ci ^{32}P one hour before autopsy (7). The activity of the glands was estimated as counts-per-minute divided by the square root of gland weight multiplied by the square root of body weights. These data were subjected to a logarithmic transformation (8). The expression of activity, therefore, was:

$$\text{Antilog } L_{10}\left(\frac{CPM}{\sqrt{\text{Gland wgt.}}} \times \sqrt{\text{Body wgt.}}\right) \quad (1).$$

This statistical procedure did not significantly alter the relative mean values for the different treatment groups from those originally observed but greatly reduced the variation within groups. The lithosperm employed in this study was a cold-water extract of finely ground roots of Lithospermum ruderale. After centrifugation and filtration, the extract was injected subcutaneously in amounts equivalent to 5.0 mg, 10.0 mg, or 20.0 mg daily of the starting material. The volume for the lithosperm treated birds was 1.0 ml daily but the assay chicks received ^{32}P and pituitary suspensions each in a volume of 0.2 ml.

Results and Discussion. Table 1 summarizes the data for the donor birds. Body weights decreased slightly at the 10.0 mg and 20.0 mg dosages but the maximum difference was non-significant ($t = 1.9360$, $P < 0.06$). Comb weights were extremely variable. There was a decrease in weight at the 20.0 mg dosage but this was non-significant ($t = 1.9658$, $P < 0.06$). Testes weights and thyroid weights were significantly decreased at the 10.0 dosage ($t = 3.312$, $P < 0.002$ and $t = 4.0147$, $P < 0.0004$ respectively). The depression was greater at the 20.0 mg dosage. There was little or no indication of lithosperm toxicity on the basis of body weights. The weights of the testes and thyroids, on the other hand, indicate that the endogenous gonadotropins and thyrotropin were ineffective at the higher dosages of lithosperm.

The differences in the assay values for anterior pituitary gonadotropins are presented in Table 2. The gonadotropin content of the pituitaries of the birds which received 5.0 mg and 10.0 mg of lithosperm was greater than that of the controls. A dose-response line for the controls was calculated and from the equation for this line equivalent potencies were determined. The potency

TABLE 1

Comparison of 46-day-old controls with 46-day-old cockerels which received 5.0 mg, 10.0 mg, or 20.0 mg of lithosperm daily for nine days, f = 16, N = 64.

	Body wgt.	Gonad wgt.	Thyroid wgt.	Comb. wgt.
Controls				
$\bar{x}$	421.04 gm[a]	132.40 mg	23.04 mg	1.62 gm
Range	450.40-393.57[b]	142.69-122.85	25.07-21.17	1.87-1.41
CV	2.09%[c]	2.88%	5.05%	3.62%
5.0 mg Lithosperm				
$\bar{x}$	424.59 gm	129.83 mg	23.48 mg	1.61 gm
Range	442.24-407.63	148.88-113.21	25.28-21.80	2.02-1.28
CV	1.26%	5.28%	4.41%	5.86%
10.0 mg Lithosperm				
$\bar{x}$	398.65 gm	110.01 mg	18.80 mg	1.58 gm
Range	414.32-383.61	120.71-100.26	20.11-17.58	1.91-1.31
CV	1.21%	3.70%	4.30%	4.80%
20.0 mg Lithosperm				
$\bar{x}$	388.66 gm	109.46 mg	16.78 mg	1.33 gm
Range	411.24-367.34	124.37-96.33	18.76-15.03	1.66-1.07
CV	1.78%	5.10%	7.34%	5.71%

a = mean, b = range, 95% confidence limits, c = coefficient of variation.

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of the pituitaries from 5.0 mg donors was equivalent to 4.86 mg, or three times that of the controls. The 10.0 mg donor pituitaries had a potency equivalent to 42.77 mg. This was 26.7 times greater than that of the control. It was observed that testis weights were not decreased in the donors given the 5.0 mg dosage but that a significant decrease occurred in the 10.0 mg donors. Synthesis obviously continued in the 10.0 mg group but gonadotropin release was blocked. The pituitaries from the 20.0 mg donors add further insight into the inhibitory action of lithosperm. The relative potency was 0.38 mg or only 23.8% of the controls. It was observed that the testes weights of the donor birds were depressed, therefore, both release and synthesis of gonadotropins clearly was inhibited by the highest dosage.

Table 3 presents the data for the thyrotropin assay. The titer of thyro-

TABLE 2

^{32}P uptake as a measure of gonadotropins in anterior pituitary glands of 46-day-old cockerel controls and 46-day-old cockerels given 5.0 mg, 10.0 mg, or 20.0 mg of lithosperm daily for nine days; f = 12, N = 144.

		0.8 mg	1.6 mg	3.2 mg
A. Control Donors	$\bar{x}$	118.21[a]	126.88	141.56
	Range	128.18-109.00[b]	138.06-116.60	155.41-128.96
	CV	2.67%[c]	2.74%	2.97%
B. Lithosperm Treated Donors				
5.0 mg	$\bar{x}$	154.42	138.02	153.58
	Range	172.14-138.52	161.75-117.77	170.06-138.69
	CV	3.39%	5.07%	3.18%
10.0 mg	$\bar{x}$	182.84	189.26	221.16
	Range	199.64-167.84	223.75-160.99	253.32-193.07
	CV	2.66%	3.19%	3.96%
20.0 mg	$\bar{x}$	94.94	110.35	115.53
	Range	104.63-86.15	120.55-101.01	127.58-104.61
	CV	3.36%	2.96%	2.29%

a = mean, b = range, 95% confidence limits, c = coefficient of variation.

Analysis of Variance (excluding control)

Treatments	11	1.5191	MSQ	F
Donors	3	1.3693	0.4564	86.1195
Doses	2	0.0963	0.0482	9.0849
Interaction	6	0.0535	0.0089	1.6824
Error	132	0.6933	S^2 = 0.0053	

H_2O Control

$\bar{x}$	77.87
Range	88.02-68.89
CV	4.43%

Equation of Line L_{10} (Dose x 10)

$\hat{Y}$ = 1.9523 + 0.1031X

	MSQ	F	P
SLOPE	0.0368	10.5088	< 0.0026
SCATTER	0.0005	0.1429	(NS)
ERROR	S^2 = 0.0035		

Equivalence of potencies of anterior pituitaries compared with 1.6 mg Control. 5.0 mg Donor $\bar{x}$ ≃ 4.86 mg, 10.0 mg Donor $\bar{x}$ ≃ 42.77 mg, 20.0 mg Donor $\bar{x}$ ≃ 0.38 mg.

tropin was less affected by lithosperm than was that of gonadotropin. There was, however, a parallel in the responses. The 5.0 mg dosage was accompanied by a pituitary thyrotropin content equivalent to the control - an estimated 1.72 mg compared with 1.60 mg. The 10.0 mg dosage again increased the level of hormone. The estimated quantity of thyrotropin was 4.20 mg, an increase of 262.5%. The 20.0 mg dosage decreased the thyrotropin titer to an 0.83 mg equi-

TABLE 3

^{32}P uptake as a measure of thyrotropin in anterior pituitary glands of 46-day-old cockerel controls and of 46-day-old cockerels given 5.0 mg, 10.0 mg, or 20.0 mg of lithosperm daily for nine days; f = 12, N = 144.

			0.8 mg	1.6 mg	3.2 mg
A.	Control Donors	$\bar{x}$	69.78[a]	89.03	103.34
		Range	78.71-61.87[b]	103.11-76.87	107.32-99.51
		CV	4.47%[c]	5.15%	1.28%
B.	Lithosperm Treated Donors				
	5.0 mg	$\bar{x}$	75.70	86.63	104.20
		Range	81.32-70.47	101.21-74.14	119.35-90.99
		CV	2.60%	5.49%	4.60%
	10.0 mg	$\bar{x}$	93.95	107.31	144.75
		Range	105.43-83.72	127.93-90.02	166.27-126.02
		CV	3.99%	5.91%	4.38%
	20.0 mg	$\bar{x}$	65.66	74.93	74.43
		Range	73.50-58.66	82.39-68.15	81.57-67.93
		CV	4.24%	3.46%	3.34%

a = mean, b = range, 95% confidence limits, c = coefficient of variation.

Analysis of Variance (excluding control)

			MSQ	F
Treatments	11	1.2713		
Donors	3	0.7299	0.2433	34.7571
Doses	2	0.4563	0.2282	32.5929
Interaction	6	0.0851	0.0142	2.0262
Error	132	0.9186	S^2 = 0.0070	

H_2O Control

$\bar{x}$	64.37
Range	71.26-58.16
CV	3.84%

Equation of Line L_{10} (Dose x 10)

$\bar{Y}$ = 1.6387 + 0.2418X

	MSQ	F	P
SLOPE	0.1377	18.1184	< 0.0001
SCATTER	0.0007	0.0877	(NS)
ERROR	S^2 = 0.0076		

Equivalence of potencies of anterior pituitaries compared with 1.6 mg Control. 5.0 mg Donor $\bar{x}$ ≃ 1.72 mg, 10.0 mg Donor $\bar{x}$ ≃ 4.20 mg, 20.0 mg Donor $\bar{x}$ ≃ 0.83 mg.

valent, or 51.9% of control. The differences in the gonadotropin and thyrotropin responses can be compared by observing the data for the analysis of variance for these hormones. The effect of "donors" had an F-value of 86.1195 for gonadotropins and an F-value of 34.7571 for thyrotropin. The "dosage" effect gave an F-value of only 9.0849 for gonadotropins but a greater F-value

of 32.5929 for thyrotropin. The ^{32}P assay in the chick does not distinguish between FSH or LH and the lower "dosage" effects may reflect changes in the relative quantities of these hormones in the pituitary. The fact that the quantities of thyrotropin and the gonadotropins are different is interesting. This indicates that the action of lithosperm is not identical for all the tropic hormones. This also was observed when exogenous hormones were administered with certain polyphenolic fractions of lithosperm (1).

It has been reported that the gonadotropin content of anterior pituitaries of mice fed lithosperm was not decreased (9,10). Improved methods of lithosperm administration and more efficient assay procedures may explain the difference between our observations and the earlier reports. Our data demonstrate unequivocally that anterior pituitary titers of gonadotropins and of thyrotropin were altered by lithosperm treatment in chicks. The quantities of the hormones were correlated with the dosage of lithosperm. The mechanism of lithosperm action is speculative. The hypothalamic releasing hormones may be inhibited or the cells of the anterior pituitary may be rendered less responsive to stimulation (11,12,13) or both may occur.

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