Effects of pinealectomy and of a bovine pineal extract in rats[1]

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WURTMAN, RICHARD JAY, MARK D. ALTSCHULE AND UNO HOLMGREN. *Effects of pinealectomy and of a bovine pineal extract in rats.* Am. J. Physiol. 197(1): 108–110. 1959.—Fifty-four 26-day-old female rats of the CD strain were divided into four groups of 10 or 11 each, i.e. *1*) controls; *2*) animals given 0.3 ml of a protein-free bovine pineal extract daily intraperitoneally for 28 days; *3*) sham-operated animals; *4*) pinealectomized animals. In a second experiment two groups of six each were divided into *a*) control animals given a diet restricted with respect to Na, K and phosphate, and *b*) animals on the same diet, given 1.0 ml/day of the pineal extract. Pinealectomy caused ovarian hypertrophy ($P < .001$), pituitary hypertrophy ($P < .001$), and adrenal hypertrophy ($P < .05$). The extract reversed these changes. Giving the extract to intact animals caused effects varying with the dose. The dose of 0.3 ml given daily caused gonadal atrophy ($P < .05$), pituitary atrophy ($P < .001$) and insignificant adrenal activity. The dose of 1.0 ml caused further gonadal atrophy ($P < .001$) and significant adrenal atrophy ($P < .05$).

SEVERAL STUDIES OF PINEAL PHYSIOLOGY which presented data adequate for statistical analysis have appeared in the past few years; almost all these studies involved only the relations between the pineal body and the ovaries of the rat. In 1954, Kitay (1) reviewed the scanty literature up to that time and showed that under certain conditions, pinealectomy resulted in ovarian hypertrophy; later that year, Kitay and Altschule (2) reported that administration of a crude bovine pineal extract for 2 weeks caused a decrease in ovarian weight and histologic evidence of retarded ovarian function. One purpose of the present work is to repeat these experiments using a much purified protein-free pineal extract (3).

Moreover, no statistically significant data are available that deal with the relations between the pineal body and other endocrine organs. Another purpose of the present work is therefore to study these relations; an experiment therefore was begun here to provide information regarding possible functional relations between the pineal and the adrenals, the anterior pituitary, and other target organs. Kitay's (1) experiments were also repeated, both as a general control in the present experiment and also to ascertain whether the ovarian changes he described after pinealectomy could be reversed by administration of a pineal extract.

Received for publication January 26, 1959.

[1] This work was aided by a grant from the Commonwealth Fund.

MATERIAL AND METHODS

Fifty-four 26-day-old female rats of the Charles River CD Strain[2] were divided into five groups of 10 or 11 each (table 1): *1*) control animals; *2*) animals given 0.3 ml/day intraperitoneally of a protein-free pineal extract (hereafter designated BPE) made essentially as described by Altschule (3); *3*) sham-operated animals, i.e. animals on which all the steps of pinealectomy were performed except the actual removal of the gland; *4*) pinealectomized animals, pinealectomized by the technique described by Kitay and Altschule (4), except that the bone flap was lifted posteriorly; *5*) pinealectomized animals given 0.3 ml/day of BPE starting 1 day after the operation.

Twenty-eight days later, i.e. when they were 54 days old, all the animals were killed and autopsied. Their ovaries, adrenals and anterior pituitaries were weighed and examined histologically; their hypothalami, thyroids, parathyroids and pineals, where present, were also examined histologically.

In addition 12 similar female rats were divided into two groups of six each (table 3). *a*) Control animals. *b*) Animals given 1.0 ml/day intraperitoneally of BPE. These animals were allowed to eat ad libitum a special diet containing no sodium, potassium or phosphate. They were also fed by gavage 1.0 ml/day of a solution containing 0.5 mEq/ml of potassium chloride and 4.0 mEq/ml of equal amounts of sodium mono- and dihydrogen phosphate; this solution was buffered to a pH of 7.0. These rats were also killed and autopsied after 28 days; their ovaries and adrenals were weighed and examined histologically, and their entire brains, including the pituitary, were fixed en bloc for staining.

The organ weights were recorded as milligrams per 100 gm of body weight. The data for each organ sepa-

[2] Obtained from the Charles River Laboratories, Boston, Mass.

TABLE 1. *Effects of Pinealectomy or BPE or Both on Rats Given Normal Diet*

Animal No.	O	A	P	O/100	A/100	P/100
Control						
22	83.5	62.4	10.6	42.4	31.7	5.38
23	79.7	60.1	12.4	38.9	29.3	6.05
29	73.1	46.1	11.2	38.1	24.0	5.81
32	76.7	65.9	10.5	35.7	30.7	4.86
39	72.8	53.3	9.8	38.9	28.5	5.24
101	40.6	46.4	8.2	24.6	28.1	4.97
102		47.6	10.6		21.6	4.82
103	67.2	58.6	9.6	35.7	31.2	5.11
104	55.6	45.2	10.0	27.8	22.6	5.00
105	51.6	40.6	7.6	27.9	22.0	4.11
106	61.8	58.4	6.8	34.3	32.7	3.83
Mean				34.43	27.49	5.01
Extract (0.3 ml)						
19	69.0	61.4	4.55	35.3	31.5	2.33
20	62.1	58.2	7.25	32.3	30.3	3.77
28	68.0	40.2	7.90	39.8	23.5	4.62
33	54.9	50.8	6.10	29.5	27.3	3.28
40	49.1	52.6	9.00	24.9	26.6	4.57
107	51.8		7.40	24.6		3.52
108		52.4	7.80		26.9	4.00
109	49.2	46.4	7.40	24.4	22.1	3.52
110	41.6	35.2	4.80	23.8	20.1	2.74
111	64.0	60.0	7.80	30.5	28.6	3.71
112	49.2	36.2	6.00	28.9	21.3	3.53
Mean				29.30	25.82	3.60
Sham-operated						
9	74.7	55.4	6.40	36.5	27.0	3.12
11	48.1	53.5	6.95	25.3	28.2	3.61
15	57.5	51.1	4.60	30.9	27.5	2.47
113	67.4	63.6	8.00	28.1	26.5	3.33
114	66.6	54.6	5.40	33.3	27.3	2.70
115	54.2	52.8	6.00	33.8	33.0	3.75
116	66.4	53.8	7.00	36.9	29.9	3.89
117	49.6	40.8	4.80	29.2	24.0	2.82
118	50.8	54.0	5.80	29.8	31.8	3.41
A	53.5	44.3	8.10	27.8	23.1	4.22
Mean				31.16	27.83	3.33
Pinealectomy						
6	106.0	61.9	9.9	55.8	32.6	5.21
10	81.5	64.6	8.1	44.3	35.0	4.40
16	74.8	55.3	8.4	49.2	36.4	5.53
21	85.1	57.0	8.5	39.7	26.6	3.99
24	74.18	73.4	10.1	40.6	40.5	5.48
30	105.0	75.0	11.7	43.6	31.1	4.85
34	81.7	75.0	8.7	38.9	35.7	4.11
36	60.9	54.8	11.9	33.8	30.4	6.58
B	84.7	56.2	12.1	36.2	24.0	5.15
C	74.2	54.6	7.5	38.6	28.4	3.88
D	74.8	56.2	9.7	34.2	25.7	4.45
Mean				41.36	31.49	4.88
Pinealectomy plus pineal extract (0.3 ml)						
12	60.0	63.7	10.9	35.3	30.1	6.40
13	65.4	60.1	9.8	32.1	29.5	4.83
17	53.8	47.8	8.5	32.6	28.9	5.18
18	82.0	67.1	10.6	40.0	32.8	5.15
25	60.7	55.6	5.7	30.2	27.7	2.84
26	57.5	62.5	8.3	33.6	36.5	4.89
31	68.1	61.5	10.5	30.3	27.3	4.68
35	73.6	63.2	11.7	37.2	31.9	5.64
E	53.8	48.6	10.2	26.9	24.3	5.10
F	42.2	59.7	9.6	21.1	29.9	4.80
G	49.8	61.0	8.8	28.4	24.8	5.05
Mean				31.60	29.43	4.96

O, A, P = ovaries, adrenals, pituitary, weight of organ(s) in milligrams. O/100, A/100, P/100 = weight of ovaries, adrenals, pituitary per 100 gm of animal.

TABLE 2. *Treatments Compared: Differences in Mean Weight per 100 gm, and Standard Errors of Those Differences*

Treatments Compared	Ovaries	Adrenals	Anterior Pituitaries
Extract—control			
0.3 ml	5.13±2.44*	1.67±1.76	1.42±0.31†
1.0 ml	13.2±3.7†	5.6 ±2.7*	
Pinealectomized—sham-operated	10.19±2.38†	3.66±1.76*	1.55±0.32†
Pinealectomized—pinealectomized plus extract	9.74±2.87†	2.06±1.72	−0.08±0.31

*P is less than 0.05. †P is less than 0.001.

TABLE 3. *Effects of BPE on Rats Given Diet Low in Na, K and PO_4*

Animal	O	A	O/100	A/100
Control				
251	35.2	35.6	27.5	27.8
252	41.6	40.1	34.1	32.9
253	58.0	39.4	47.5	32.3
254	47.0	48.1	39.8	40.8
255	40.5	38.5	32.4	30.8
256	49.8	38.3	46.1	35.5
Mean			37.9	33.3
Extract (1.0 ml)				
221	30.9	22.1	25.7	18.4
217	30.4	33.8	26.9	29.9
212	31.0	29.6	29.8	28.4
225	31.2	34.0	26.6	28.6
206	20.0	33.2	17.2	28.6
214	26.5	39.5	21.9	32.6
Mean			24.7	27.7

O = ovaries; A = adrenals, weight of organ in milligrams. O/100, A/100 = weight of ovaries and adrenals per 100 gm of animal.

rately were subjected to an analysis of variance (5) which yielded a 'pooled' estimate of the standard deviations between groups. This value for s was used in calculating standard errors for individual means and for differences between means. The observed difference between mean values for any two groups was evaluated by the t-distribution of the ratio of the difference to its standard error.

Animals to which BPE was administered were compared with unoperated controls, while animals that had been pinealectomized, regardless of whether they had been given BPE, were compared with sham-operated controls.

OBSERVATIONS

Ovaries. Pinealectomy resulted in ovarian hypertrophy ($P < .001$) (tables 1, 2). The administration of BPE resulted in decreased ovarian weight, the decrease varying with the dose. Administration of 0.3 ml/day caused a decrease of 15% ($P < .05$); administration of 1.0 ml/day caused a decrease of 35% ($P < .001$). Daily administration of 0.3 ml of BPE reversed the ovarian hypertrophy generally caused by pinealectomy ($P < .001$) (tables 1, 2 and 3).

Adrenals. Pinealectomy resulted in adrenal hypertrophy ($P < .05$) (tables 1, 2). The administration of 0.3 ml of BPE to both control and pinealectomized animals resulted in a small decrease (7%) in adrenal weight, which was not statistically significant (tables 1, 2). When 1.0 ml was administered to sodium-, potassium-, and phosphate-restricted animals which were compared to similarly restricted controls, the decrease in adrenal weight was 17%, and was statistically significant ($P < .05$) (table 3).

Anterior pituitary. Pinealectomy reversed ($P < .001$) the decrease in pituitary weight caused by sham-operation (tables 1, 2). BPE caused a great decrease in pituitary weight ($P < .001$), when the animals to which extract was administered were compared to normal controls (tables 1, 2 and 3). Administration of BPE to pinealectomized animals did not, with the dose used, result in an inhibition of the relative hypertrophy caused by pinealectomy (tables 1, 2).

DISCUSSION

As Kitay (1, 2) showed previously, pinealectomy causes ovarian hypertrophy, and administration of BPE results in retarded ovarian growth. The changes in organ weight are clearly independent of such changes in body weight which might have occurred as a result of pinealectomy. The ovarian hypertrophy caused by pinealectomy was prevented by administration of BPE in the present study. Pinealectomy also caused adrenal and anterior pituitary hypertrophy, and giving BPE caused retardation of growth of both glands. The data indicate that the inhibitory effects of BPE on the weights of the ovary and the adrenal, at least, follow a dose-response relationship.

It therefore appears that the pineal body has endocrine relationships with at least the ovary, the adrenal and the anterior pituitary. Moreover, the pineal appears to be unique among endocrine organs in that it inhibits the growth and perhaps some of the functions of all those of its target organs studied here. The question of the specificity of the effects of BPE on these other glands is an important one. The extracts used in the present study contained 8 or 10 mg of organic solids per ml (plus 4 mg of sodium chloride). The effects caused by such small amounts of material, i.e. about 3 mg/day, suggests that a highly potent material was being injected. Moreover, the fact that the administration of BPE reversed most of the effects of pinealectomy support the view that the action of BPE is specific.

There should be no need to defend the comparison of pinealectomized and sham-operated rather than intact animals in the present work. The operation of pinealectomy must have a nonspecific stressing effect, and the only valid comparison that can be made is with sham-operated animals. The present data showed that pinealectomy and the sham operation both caused reductions in pituitary weight. (Of course the effect of pinealectomy was only a small absolute decrease in pituitary, i.e. an increase relative to the effect of the sham operation.)

The present study does not necessarily demonstrate that the pineal elaborates a single factor whose absence causes the changes in the different glands studied here. Further studies on pineal physiology are in progress, and reports on them and on the histologic data obtained from this experiment are in preparation.

We are indebted to Dr. Mindel C. Sheps of the Department of Preventive Medicine, Harvard Medical School, for her assistance and advice in the statistical analysis of these data.

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