

Effective Stimulation of Crop-Sacs by Prolactin in Hypophysectomized and in Adrenalectomized Pigeons.

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Four years ago 2 of us¹ reported the stimulation of the crop-sacs of one completely (and of another incompletely) hypophysectomized pigeon with prolactin. Subsequently this result was confirmed by other tests which were not published. Recently Gomez and Turner² reported failure to obtain such stimulation, even with the sensitive local crop-sac method, on 16 completely hypophysectomized pigeons. Following acquaintance with their results our own series of unreported tests was increased sufficiently to provide a quantitative measure of the effect of hypophysectomy on the crop-sac response. These and other data relating to this response after hypophysectomy or adrenalectomy are presented here.

Hypophysectomy was done by the parapharyngeal route which permits the removal of the anterior pituitary with but little loss of posterior lobe tissue in most cases. Completeness of removal of anterior pituitary tissue (or of other extirpated tissue) was investigated with serial sections in all cases. Intramuscular injection only was employed. The chief criterion of crop-sac stimulation was its increased weight; but crop-milk formation was observed in most of the much prolonged tests as well as in slightly more than half the 4-day tests, and histological proof of active cell division (including colchicine test) was observed in the few cases thus studied at the end of the usual 4-day term of injection. Common pigeons of various races (and ages) were used. The "control" crop-sac weights listed in Table I are average values of unstimulated crop-sac areas obtained for groups (4 to 14) of unoperated birds of the same race.

The data of Table I make it fairly evident that when less than 5% of anterior pituitary tissue is present the crop-sac response (increase in weight) is hardly different from that in complete hypophysectomy; and our own fuller information indicates that the factor of *race*—together with the marked difference in body weights—is

¹ Riddle, O., Bates, R. W., and Dykshorn, S. W., *Am. J. Physiol.*, 1933, **105**, 191.

² Gomez, E. T., and Turner, C. W., *PROC. SOC. EXP. BIOL. AND MED.*, 1936, **35**, 59.

CROP-SAC STIMULATION IN HYPOPHYSECTOMIZED PIGEONS 409

TABLE I.
Growth Response in Crop-Sacs of Hypophysectomized (and Other Operated) Adult Pigeons to 4 Daily Injections of Prolactin.

No. of bird	Age when killed, mo.	Time since operation, days	Body wt., gm.	Prolactin daily, units	Weight of crop-sacs	
					Control, mg.	Found, mg.
Hypophysectomy complete.						
1	16.3	177	530	30	1265	4800
2	26.0	191	428	30	1032	4700*
3	20.0	8	361	30	890	2755
4	16.2	8	298	30	800	2830
5	32.4	8	547	30	1265	4145
6	7.8	14	436	30	950	2865
7	8.8	14	326	30	726	2855
Less than 1% pituitary present.						
8	17.1	8	516	30	1265	5475
9	9.5	8	426	30	925	5215
10	7.7	14	449	30	1050	2200
11	19.9	111	454	30	1025	6070
Less than 5% pituitary present.						
12	28.8	170	264	30	726	5300†
13	18.1	287	374	30	1012	3915
14	27.5	162	572	5	1350	1665
15	20.6	116	280	5	726	895
Adrenalectomized only.						
16	18.0	8	517	30	1300	7570
17	2.8	6	588	30	1300	5380‡
Thyroidectomized only.						
18	33.0	180	279	15	630	4060
19	29.0	30	510	50	1025	17620§

*This bird also completely castrated 58 days before.

†Injected for 7 days (giving greater response than 4 days).

‡A trace of adrenal present on right side.

§Fragment (6.8 mg.) of one thyroid present; dosed with prolactin, thyrotropic and F.S.H. for 9 days.

chiefly responsible for the wide variation in the values obtained in birds Nos. 1 to 13. When we calculate¹ the crop-sac response of 12 of these birds (No. 12 omitted because injected for 7 days), each of which was injected with a total of 120 prolactin units, it is found that the weight of their crop-sacs correspond to only 63 units. Moreover, these 63 units must be divided by 4, since these crop-sac weights were obtained in *adults*—whose response averages about 4 times greater than that of the immature birds utilized in all original prolactin assays. Thus the average crop-sac weight obtained in these adult hypophysectomized pigeons is that expected to result from only 16 instead of 120 prolactin units; in other words, this response in the crop-sac of the hypophysectomized pigeon is only about one-eighth that of the normal mature pigeon. Injections were begun in this series of birds at periods ranging from 4 to 283 days after hypophysectomy.

It is clear that the crop-sacs of hypophysectomized pigeons are

410 CROP-SAC STIMULATION IN HYPOPHYSECTOMIZED PIGEONS

effectively stimulated by prolactin; and equally clear that very much more prolactin is required to give a particular amount of proliferation than in the intact pigeon. Some impairment of crop-gland structure by this operation is scarcely a matter of surprise; it is still to be shown that there is any organ of the body which remains unaffected by complete removal of the anterior pituitary. We suppose that the quantity of prolactin injected (intramuscularly or intradermally) by Gomez and Turner was too small to give them a definite response in their pituitaryless birds.

Table I also lists data indicating that the crop-sacs of an adult adrenalectomized pigeon respond to prolactin when dosage was begun 4 days after this operation. For a completely thyroidectomized pigeon a like result was obtained from dosage beginning 176 days after thyroid removal.

TABLE II.
Growth Response in Crop-sacs of Very Young Completely Hypophysectomized Pigeons Dosed During 2 to 38 Days with Various Quantities of Prolactin.

No. of bird	Age (after hatching)		Body wt., gm.	Dosage		Wt. of crop-sacs found, mg.
	When operated, days	When killed, days		Prolactin daily, units	Duration, days	
20	12	29	230	25	18	2155
21	10	22	152	25	11	1440*
22	13	35	183	25	20	1415*
23	14	23	126	25	2½	970
24	12	28	170	25	4	710
25	8	21	116	6	12	885*
26	10	27	191	2	17	2705*
27	8	33	147	6	22	1280*
	Whole A.P. extract injected (prolactin content known).					
28	17	30	170	25	13	2435
29	12	21	152	25	9	2225
30	8	18	187	12	5	2780*
31	16	58	215	12	38	2150*
32	8	31	298	10	23	8510*

*More or less secretion present.

The data of Table II demonstrate that after removal of the pituitary from very young pigeons their crop-sacs respond to prolactin by growth and secretion. In all of the 13 tests—including the small crop-sacs (and short-term dosage) of Nos. 23 and 24—effective stimulation was obtained. In these tests dosage was started 0 to 12 days after operation and continued from 2 to 38 days. The daily dosage varied between 2 and 25 units. These variables of time, dosage, age and size provide data of some additional interest though we cannot supply control crop-sac weights (relatively larger in young than in adults) for these young squabs. In somewhat older,

and in adult, pigeons usual dosages of prolactin are continued during about 4 to 6 days before the secretion of crop-milk begins. The tabulated data show that under small or moderate dosage (2 to 12 units daily) these very young hypophysectomized birds may begin crop-milk secretion within as little as 5 days, and they may also secrete at least as late as the 38th day. The effect of long-continued or of excessive stimulation of the crop-sacs of either normal or hypophysectomized pigeons is otherwise quite unknown.

Summary. In all of 20 pigeons completely hypophysectomized from 1 to 287 days earlier the crop-sacs were stimulated by prolactin to proliferation, and often to crop-milk formation within 4 days. These complete responses were obtained in both very immature and in adult individuals. Quantitative measurements made on the operated adults show, however, that their crop-sac response is only about one-eighth that of unoperated mature pigeons.

Complete adrenalectomy did not prevent the usual response of the crop-sacs to prolactin in a test started 4 days after operation. Thyroidectomy did not significantly affect the usual crop-sac response to prolactin in a pigeon operated 176 days earlier.

9250

Erythrocyte Count in Four Inbred Strains of Mice.

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Since wide variation in the erythrocyte count for adult mice (*Mus musculus*) appears in the literature, the problem of whether this variation is genetic arose. It also seemed desirable to establish a mean red cell count on a more adequate number of animals than usually reported.

The figures given by various authors for erythrocyte counts (in millions per cu. mm.) are as follows: Simmonds,¹ 6 to 8; Mixer and Hunt,² 9.7 for "non-flexed" based on 12 animals and 11.2 for "flexed" based on 16 animals; Kamenoff,³ 9.9 for "non-flexed" based on 13 and 10.9 for "flexed" based on 5. The following figures are summarized from Scarborough⁴ (in millions per cu. mm.):

¹ Simmonds, J. P., *Anat. Record*, 1925, **30**, 99.

² Mixer, R., and Hunt, H. R., *Genetics*, 1933, **18**, 367.

³ Kamenoff, R. J., *J. Morph.*, 1935, **58**, 117.

⁴ Scarborough, R. A., *Yale J. Biol. and Med.*, 1930, **3**, 272.