if anterior hypophyses were grafted together with ovaries. Transplantation of anterior hypophyses alone failed to produce breast cancer. No mammary tumors occurred in castrates into which ovaries, anterior hypophyses alone or anterior hypophyses in combination with ovaries were grafted at 7 months of age. Thus in male mice, an age factor which is independent of the testicle operates in the production of breast cancer by ovarian and anterior hypophyseal grafts.

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Effects of Restricted Feed Intake in Intact and Ovariectomized Rats on Pituitary Lactogen and Gonadotrophin.*

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Starvation generally has been shown to result in decreases in the weights and functions of the endocrine glands, and these decreases have been attributed to a reduction in secretory activity by the anterior pituitary.[1-6] The adrenals represent an exception, since in cases of extreme or total starvation their weight and activity are actually augmented rather than reduced.[1,7,8]

Assays of the gonadotrophin content of the pituitaries of starved rats have revealed a decrease in the hands of some workers,[4,5] while others have reported no change in pituitary content.[9,10] The lactogenic hormone content of the pituitary was found to be reduced during complete starvation in lactating rats.[11]

It was the purpose of these experiments to seek further clarification of the effects of restricted food intake on the pituitary content of these two hormones, since under other conditions the latter has served as a reliable index of the amounts of these hormones present in the blood stream.[10-13] It was also considered desirable to determine the above effects in both intact and ovariectomized rats, since in the latter the pituitary gonadotrophin content is known to be higher[14-16] and pituitary lactogen lower than in intact rats.[13,17]

Methods. Forty-eight intact female rats of about 200 g body weight and 35 rats which had been ovariectomized about a month previous and weighed about 230 g were used in

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1 Mulinos, M. G., and Pomerantz, L., *J. Nutrition*, 1940, **19**, 493.

2 Mulinos, M. G., and Pomerantz, L., *Endocrinology*, 1941, **29**, 558.

3 Mulinos, M. G., and Pomerantz, L., *Endocrinology*, 1941, **29**, 267.

4 Mason, K. E., and Wolfe, J. M., *Anat. Rec.*, 1930, **45**, 232.

5 Werner, S. C., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 101.

6 Stephens, D. J., and Allen, W. M., *Endocrinology*, 1941, **28**, 580.

7 Mulinos, M. G., and Pomerantz, L., *Am. J. Physiol.*, 1941, **132**, 368.

8 Boutwell, R. K., Brush, M. K., and Rusch, H. P., *Am. J. Physiol.*, 1948, **154**, 517.

9 Marrian, G. F., and Parkes, A. S., *Proc. Roy. Soc. London*, 1929, **105**b, 248.

10 Maddock, W. O., and Heller, C. G., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 595.

11 Meites, J., and Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bul.* 416, 1948.

12 Meites, J., and Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 190.

13 Meites, J., and Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bul.* 415, 1948.

14 Engle, E. T., *Am. J. Physiol.*, 1929, **88**, 101.

15 Evans, H. M., and Simpson, M. E., *Am. J. Physiol.*, 1929, **89**, 371.

16 Wolfe, J. M., *Am. J. Anat.*, 1932, **50**, 351.

17 Reece, R. P., and Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bul.* 266, 1937.

TABLE I. Weights of Endocrine Glands of Intact and Ovariectomized Rats on Restricted Feed Intakes.

No. rats per gp.	Procedure	Orig. body wt, g	Final body wt, g	% diff. in body wt	Ovarian wt per rat mg	Ovarian wt per 100 g body wt mg	Uterine wt per rat mg	Uterine wt per 100 g body wt mg	Adrenal wt per rat mg	Adrenal wt per 100 g body wt	Thyroid wt per rat mg	Thyroid wt per 100 g body wt	Pituitary wt per rat mg	Pituitary wt per 100 g body wt
	Intact Rats.													
10	Controls fed *ad lib.*	196	220	+12	62.4	28.4	393.4	179.0	63.2 ± 1.8*	28.8	18.1 ± 0.9*	8.2	15.2 ± 1.2*	6.9
10	Fed ¾ *ad lib.*	193	197	+ 2	52.3	26.6	374.5	190.4	54.0 ± 2.5	27.5	15.9 ± 0.9	8.1	11.7 ± 0.6	6.0
10	,, ½ ,, ,,	197	173	—12	50.5	29.1	256.0	147.7	47.3 ± 2.0	27.3	12.6 ± 0.6	7.3	10.9 ± 0.4	6.3
9	,, ¼ ,, ,,	200	153	—24	46.7	30.5	154.3	100.9	47.7 ± 3.0	31.2	13.0 ± 0.5	8.5	9.1 ± 0.2	6.0
9	No food	209	141	—33	51.1	36.2	184.0	130.5	61.8 ± 3.6	43.8	10.6 ± 0.2	7.5	8.9 ± 0.4	6.3
	Ovariectomized Rats.													
7	Controls fed *ad lib.*	230	248	+ 8					57.8 ± 3.0	23.1	21.9 ± 1.6	8.8	11.3 ± 0.8	4.6
7	Fed ¾ *ad lib.*	230	221	— 4					51.3 ± 1.9	23.2	16.1 ± 1.2	7.3	13.2 ± 0.7	5.9
7	,, ½ ,, ,,	228	194	—15					48.0 ± 3.3	24.7	15.8 ± 1.7	8.1	11.8 ± 0.5	6.1
7	,, ¼ ,, ,,	226	160	—30					52.3 ± 2.4	32.7	15.0 ± 1.1	9.4	11.1 ± 0.6	7.0
7	No food	234	162	—30					60.9 ± 1.6	37.6	16.8 ± 1.4	10.4	11.7 ± 0.5	7.2

* Standard error of the mean $= \sqrt{\frac{\Sigma d^2}{n(n-1)}}$

these experiments. All were of the Sherman strain. The intact and ovariectomized rats were divided into 5 groups each, and were put on *ad libitum*, ¾, ½, ¼ and no-feed regimes. The ¾, ½ and ¼ feed levels were determined daily from the *ad libitum* intake of the control group. The *ad libitum* and partially-fed groups were killed at the end of 14 days and the unfed group at the end of 7 days. The endocrine glands were removed, trimmed free of extraneous tissue, and weighed on a Roller-Smith balance.

The pituitaries from each group were macerated into a fine paste with a small mortar and pestle. Distilled water was then added to a volume of one ml per pituitary, and the fine suspension was taken up with a syringe through a number 25 needle.

Gonadotrophic potency was measured by injecting the equivalent of 5 pituitaries from each group into 5 immature female rats, making two injections daily for 5 days. On the 6th day the rats were killed and the ovaries and uteri were weighed. Lactogenic potency was assayed by injecting the equivalent of 2 pituitaries from each group intradermally over the crop glands of 5 mature White Carneau pigeons during a 4-day period, and rating the crop responses by the Reece-Turner[17] method on the 5th day.

Results. Although incidental to the main problem, the effects of restricted feed intake on the weights of the endocrine glands will be considered first (Table I). In the intact rats, the weights of the pituitary, thyroid, adrenals and ovaries were reduced on an individual basis in all the restricted diet groups, with the exception of the adrenal weights of the completely starved group. On a 100 g body weight basis, the weights of these glands remained the same, again with the exception of the adrenal weights of the completely starved group. These results agree on the whole with those previously reported by other workers.

In the ovariectomized rats, there was no loss of pituitary weight in any of the restricted diet groups; a slight but similar loss of thyroid weight in all restricted diet groups; and a slight loss of adrenal weight in all restricted diet groups except the non-fed group.

TABLE II. Assays of the Gonadotrophin and Lactogen Content of Pituitaries of Intact and Ovariectomized Rats on Restricted Feed Intakes.

Intact Donor Rats Procedure	Avg pit. wt, mg	Body wt, g	Recipient rats* (gonadotrophin assays) Ovarian wt per rat	Ovarian wt per 100 g body wt	Uterine wt per rat	Uterine wt per 100 g body wt	Recipient pigeons‡ (lactogenic assays) Avg Reece-Turner units per pit.	units per mg pit.
Controls, fed *ad lib.*	15.2	77	18.6 ± 2.3†	24.2	53.3 ± 2.5†	69.5	4.5	0.30
Fed ¾ *ad lib.*	11.7	77	19.2 ± 2.5	25.0	71.9 ± 12.0	93.8	4.0	0.34
,, ½ ,, ,,	10.9	75	17.8 ± 2.2	23.8	72.4 ± 9.2	87.0	3.0	0.28
,, ¼ ,, ,,	9.1	78	20.0 ± 2.3	22.7	88.6 ± 3.9	92.5	2.5	0.27
No feed	8.9	81	17.8 ± 2.4	24.8	65.0 ± 9.1	110.0	2.2	0.25
Controls uninjected		74	15.2 ± 1.8	20.5	23.4 ± 4.6	31.0		
Ovariectomized Donor Rats								
Controls, fed *ad lib.*	11.3	68	65.7 ± 6.2	96.3	84.4 ± 5.0	123.8	3.1	0.27
Fed ¾ *ad lib.*	13.2	74	63.5 ± 11.8	85.8	116.0 ± 7.0	158.0	3.1	0.23
,, ½ ,, ,,	11.8	71	59.6 ± 7.2	84.1	85.0 ± 10.0	120.0	2.5	0.21
,, ¼ ,, ,,	11.1	73	65.6 ± 5.5	90.1	99.4 ± 5.4	136.5	1.8	0.16
No feed	11.7	72	65.7 ± 6.2	96.3	84.4 ± 5.0	123.8	1.8	0.15

* The equivalent of one donor rat pituitary was injected into each recipient rat.
‡ The equivalent of two donor rat pituitaries was injected into 5 pigeons.
† Standard error of the mean = $\sqrt{\frac{\Sigma d^2}{n(n-1)}}$

On a 100 g body weight basis, pituitary weight was increased in all restricted diet groups; thyroid weight was slightly reduced on the ¾ and ½ feed regimes but increased on the ¼ and no-feed regimes; and adrenal weight was unchanged on ¾ and ½ feed intake and increased on ¼ and no-feed intake.

These differential effects of restricted food intake on endocrine weights in intact and ovariectomized rats are interesting since they have not been reported previously. This may be due to the fact that almost all of the previous work on this problem has been done in intact animals. It is not known whether such differential effects would be obtained in intact and ovariectomized rats during more prolonged periods of feed restriction.

The effects of reduced feed intake on the lactogenic and gonadotrophic hormone content of the pituitaries are given in Table II. In neither the intact nor ovariectomized rats on restricted feed allowances was the gonadotrophin content of the pituitaries reduced from that of the controls fed *ad libitum*. In fact the concentration was actually increased on the basis of hormone content per milligram of pituitary in the intact groups (since pituitary weight was decreased on the restricted diets). The pituitaries of the ovariectomized rats contained about 10 times as much gonadotrophin as the intact rats, as indicated by their ability to increase ovarian weight in the recipient immature rats.

The lactogenic potency of the pituitaries of both intact and ovariectomized rats on reduced feed was decreased below that of the controls fed *ad libitum*, on an individual basis. The decrease in lactogenic hormone was roughly proportional to the decrease in feed intake. On the basis of hormone concentration per milligram of pituitary there was only a slight decrease in the intact groups and a marked decrease in the ovariectomized groups. In corroboration of previous findings[13,17] the lactogen content of the pituitaries of the ovariectomized control rats was reduced below that of the intact controls.

Discussion. Maddock and Heller[10] have suggested a possible cause for the divergent results reported by different workers on the

assay of pituitary gonadotrophin content in starved rats. They pointed out that other investigators implanted entire pituitary glands into recipient assay rats, whereas they injected suspensions of pituitary tissue, as was also done in our experiments. The implantation method is believed to be less exact since necrosis, growth, or encapsulation may occur, resulting in an exceedingly variable delivery of gonadotrophic hormone.

It is not clear as to why the pituitary gonadotrophin content remains the same during periods of starvation while pituitary lactogen becomes reduced. There can be no doubt that starvation, depending on its degree, results either in a reduction or complete cessation of gonadal and mammary gland activity. This would certainly seem to indicate that the amounts of circulating gonadotrophic and lactogenic hormones are reduced.

On the assumption that pituitary content of a hormone represents the resultant between the amount being produced and the amount released into the blood stream, Maddock and Heller[10] explain the 'dichotomy' between gonadotrophin content in the pituitary and blood stream of starved rats as being due to a failure of release and to a minimum of production by the pituitary. The decrease of lactogenic hormone content in the pituitary during starvation could be explained as being due primarily to a failure of production with little or no interference with the release mechanism.

Assays of pituitary content of gonadotrophin have proven to be reliable indicators of the amounts present in the circulation following ovariectomy, during estrogen administration, but not during starvation. Assays of the lactogen content of the pituitary have accurately reflected the amounts present in the blood stream during lactation, during estrogen or testosterone administration, and also during periods of restricted feed intake.

Summary. The lactogenic and gonadotrophic hormone content of pituitaries of intact and ovariectomized rats was determined after full, ¾, ½, ¼ and no-feed regimes. The full or partially-fed groups were sacrificed at the end of 14 days and the unfed group at the end of 7 days. Pituitary lactogen content was reduced in the intact and ovariectomized rats on the ½, ¼ and no-feed regimes below that of the ¾ and full-fed controls. No change occurred in the gonadotrophin content of the pituitaries of either the intact or ovariectomized rats, regardless of the level of feed intake. In the intact but not in the ovariectomized rats, restricted feed intake caused a marked reduction in the weights of the pituitary, thyroid and adrenals, except that on the no-feed regime adrenal weight was increased in both groups.

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Assay of Secretin and Cholecystokinin Concentrates.*

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The most feasible assay of secretin concentrates involves the cannulation of the pancreatic duct and observation of the increased rate of pancreatic juice output upon intravenous injection of the sample. Ivy, Kloster, Drewyer and Lueth[1] introduced the threshold dose, namely, that amount of a secretin concentrate causing a flow of 10 drops (0.4 ml) of pancreatic juice within a 10-minute period over the control rate in the dog. In a later work, Greengard and Ivy[2] reported that the threshold dose of a preparation obtained from hog duodenums (SI) and adopted as a standard was 0.25 mg for many dogs. This

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1 Ivy, A. C., Kloster, G., Drewyer, G. E., and Lueth, H. C., *Am. J. Physiol.*, 1930, **95**, 37.

2 Greengard, H., and Ivy, A. C., *Am. J. Physiol.*, 1938, **124**, 427.