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Failure of Gonadotropic Function of the Rat Hypophysis During Chronic Inanition.*

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The disease picture of anorexia nervosa with marked weight loss has many features similar to that of true hypophyseal destruction in Simmond's disease. The similarity of the syndromes raises the possibility that the reduced food intake in anorexia nervosa may cause hypophyseal failure. This is further suggested by the amenorrhea that occurs with starvation and chronic wasting disease.

To test this hypothesis, the effect of inanition on hypophyseal function has been studied in the rat during the past 2 years. Marrian and Parkes,[1, 2] in investigating the anestrus of vitamin B deficiency in the rat, found that the ovaries of such rats were still responsive to hypophyseal implants, as were also the ovaries of rats on a severely diminished food intake. This latter has been recently confirmed by Mulinos, *et al.*[3] The presence of gonadotropic principle in the hypophysis of the vitamin B deficient rats, however, led Marrian and Parkes[1] to believe that the rôle of the hypophysis in the anestrus in this condition was not clear. The experiments described below indicate plainly that during chronic inanition, the origin of the anestrus is in the anterior hypophysis.

Adult female rats, 180-260 g in weight, with regular estrous cycles, and immature 22-day-old female rats of the Long-Evans strain, were used throughout. The rats were kept in individual cages, given water *ad lib.*, and were fed a standard rat diet, McCollum mixture No. 1, limited only in amount. Cod liver oil and yeast supplements were given twice a week. The rats were used after 1-4 months on this regime, having lost 30%-50% of their original body weight. Swiss mice, 22 days old at weaning, were used as recipients for the hypophyseal implants, with autopsy 96 hours after a single

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1 Marrian, G. F., and Parkes, A. S., *Proc. Roy. Soc. B*, 1929, **105**, 248.

2 Parkes, A. S., *Quart. J. Exp. Physiol.*, 1928, **18**, 397.

3 Mulinos, M. G., Pomerantz, L., Smelser, J., and Kurzrok, R., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 79.

implant, using the technic of Smith. Tissues were fixed and stained by the usual technics.

I. The effect of inanition on the structure and function of the ovary has been previously reported[4, 5, 6] and has been confirmed here in a small series. However, the microscopic appearance of the ovary can now be identified as that occurring after partial hypophysectomy. In the present series, the estrous cycle, ovarian and uterine weights were observed. Sixty-one of 75 adult rats showed a diestrous smear after 2 weeks, and 71 of the 75 after 3 weeks of inanition with no reappearance of estrus except in 4 rats, in 1 of which cycles never ceased despite pronounced weight loss. Four adult rats were autopsied after 1 month of inanition. Ovarian weights ranged from 26.5-53.0 mg, averaging 29 mg. Five hypophysectomized rats of about the same weight, 1 month after the operation had ovarian weights of 14.2-25.4 mg, averaging 18.2 mg. The ovarian weights of both of these groups are well below those of normal controls. This is also true of the uterine weights, which ranged from 122-162 mg (av. 141) in the starved rats compared to 76-201 mg (av. 113) in 7 castrate starved rats and 76-111 mg (av. 88) in 5 hypophysectomized rats. The above figures in animals suffering from severe inanition approach those seen after almost complete ablation of the anterior hypophysis. The gross and microscopic appearance of the ovaries, and their response to gonadotropic principles are also like those of the partially hypophysectomized rat.

II. The responsiveness of the ovary during inanition was tested with the gonadotropic principles of pregnancy urine, anterior hypophysis, and pregnant mare's serum.‡

a. Eight starved rats, 3 adult and 5 immature, were injected with pregnancy urine principle (1.5-3 r.u. total). There was some growth of follicles, thus differing from the hypophysectomized rat. There was also an occasional formation of corpora lutea and thecal luteinization. The adults remained in estrus only 48 hours.

b. The hypophyseal principle was injected into 34 adult starved rats, a total dosage of 2-20 mg being administered in 2 equal daily injections. Estrous response appeared with 10 mg dosage and

4 Jackson, C. M., *The Effects of Inanition and Malnutrition upon Growth and Structure*, P. Blakiston, Philadelphia, 1925, 616 pp.

5 Papanicolaou, G. N., and Stockard, C. R., PROC. SOC. EXP. BIOL. AND MED., 1920, **17**, 143.

6 Evans, H. M., and Bishop, K. S., *J. Met. Res.*, 1923, **3**, 233.

‡ The pregnancy urine extract, Follutein, was given by E. R. Squibb and Sons. The anterior hypophyseal extract was primarily thyrotropic and was provided by the Schering Corporation. The pregnant mare serum extract was Antex Leo.

lasted 1-2 days. Nineteen 22-day-old normal rats were given 2-40 mg in 5 doses over 36 hours and autopsied 96 hours after the first injection. Minimal ovarian and uterine weight response occurred with the 10 mg total dose. Ovaries averaged 18.2 mg, uteri 49.2 mg, compared with untreated control figures of 14.7 and 33.8 mg respectively.

c. Pregnant mare's serum gonadotropic extract (Antex) was injected into 47 adult starved rats using total doses of 0.05-0.8 mg (0.25-4.0 m.u.), and into 31 normal unstarved immature rats, 0.05-0.6 mg (0.25-3.0 m.u.) being used, and the duration of estrus observed. The material was given to the adults in 2 injections and to the immatures in 5 injections over a 2-day period. In the 11 normal immature rats receiving 0.6 mg (3 m.u.) total of extract, estrus appeared on day 4 and lasted 1 day, whereas in the adult starved rats given this dosage, estrus lasted 3-13 days, and in 10 trials on 8 hypophysectomized rats with the same dosage, it lasted for 6 days. The cessation of ovarian function during starvation therefore has been shown to occur in the face of normal or slightly increased reactivity of the ovaries to gonadotropic principles. Thus, inability of the ovary to react to gonadotropic principle from the hypophysis cannot be the cause of the failure of ovarian function during inanition. As regards duration of estrus induced by pregnant mare serum, the starved rats behaved like the hypophysectomized ones though we feel uncertain why estrus should be prolonged in either type.

III. The fact that the ovary is sensitive to the hypophyseal principle suggests that interference with hypophyseal function might be the cause of the ovarian inactivity. The hypophyses from 6 adult starved rats were examined. The histological appearance, with evidence of atrophy, described by Jackson,[4] was found. No castration changes were seen as would be expected were the ovarian failure primary and the hypophysis responding normally. Eleven rats were starved and castrated. One to 2 months after castration the hypophyses were examined. No castration changes were found in the glands of 8 of the 11. The glands were identical in appearance with those of the intact starved animals. The castration change was prevented from developing whether the operation was performed before or during the inanition. In the other 3 there were definite castration changes. The degree of change was somewhat less than in the normal fed castrate.

Following castration, there is normally a marked rise in the gonadotropic potency of the castration hypophysis.[7] Tests were

[7] Engle, E. T., *Am. J. Physiol.*, 1929, **88**, 101.

TABLE I.
Assay by Mouse and Uterus Weight of Gonadotropic Potency of Starved Rat Hypophyses.

Mouse Organ	Total Number of Donors Tested						
	Adult starved castrate	Adult normal castrate	Adult starved	Adult normal	Immature starved	Immature normal	Normal mice controls
Ovaries (wt mg)							
0.0-3.3	6	0	6	3	0	1	10
3.4-5.0	2	0	0	4	4	1	2
over 5.0	4	5	0	3	0	2	0
Range in wt	2.1-7.7 mg	5.5-11.7 mg	1.9-3.3 mg	1.4-6.9 mg	3.3-4.8 mg	2.4-5.6 mg	1.6-4.5 mg
Avg wt	3.9 mg	8.3 mg	2.6 mg	4.2 mg	3.7 mg	3.5 mg	3.0 mg
Uterus (wt mg)							
0.0-12.0	8	0	5	2	0	1	12
over 13.0	4	5	1	8	4	3	0
Range in wt	8.2-44.0 mg	23.4-50.2 mg	7.2-14.7 mg	9.1-51.6 mg	17.7-27.9 mg	7.6-46.6 mg	3.9-11.6 mg
Avg wt	18.4 mg	34.4 mg	9.8 mg	29.5 mg	21.7 mg	23.0 mg	6.8 mg
Vagina							
Closed	4	0	5	0			12
Partly open	2	0	0	0			
Open	6	5	1	6			

carried out to determine whether or not an increase in potency after castration occurred in the starved animals. The hypophyses of 6 intact starved and 12 starved rats, castrated for at least 1 month, were implanted into the thigh muscles of 22-day-old white mice, 1 hypophysis per mouse. Ten normal rats and 5 normally fed rats, castrated for 2 months, served as controls. The hypophyses of 4 immature starved, and 4 immature normally fed rats were also assayed (Table I). The mice were killed 96 hours after implantation. There was a marked decrease in the potency of the hypophyses of the intact starved rats compared with the normal controls. Eight of the 12 castrate, starved rats showed not only no increase in potency of the hypophysis but the same low content as the intact starved rats. The glands of the other 4 contained gonadotropic potency of the order of normal castrates. These results indicate a failure of the hypophysis during inanition to respond to castration. The findings of a rise in potency with castration in 4 of the 12 castrate starved rats agrees with the percentage of starved rats which showed castration changes structurally.

The evidence, then, reveals that the gonadotropic function of the hypophysis fails during chronic inanition in the rat. The ovaries shrink to a partially hypophysectomized level. They are still normally responsive to gonadotropic substances. The atrophic changes in the hypophysis described after starvation have been confirmed and associated with this is a failure of response to castration either histologically or by rise in gonadotropic titer. It seems not improbable that the same mechanism operates to produce amenorrhea in human inanition and in anorexia nervosa with marked weight loss.

The other functions of the hypophysis during chronic inanition are being investigated.