mental renal disorder by parabiosis as well as by a technic first described by Paterson (5), supports the contention that this experimental membranous glomerulonephritis is due to an autoimmune mechanism. Whether the described circulating antibodies(3) produce renal disease, or whether a delayed hypersensitivity mechanism is at play, is not clear.

Summary. 1. Fourteen pairs of parabiosed litter mates were used. In 9 of these autoimmune nephrosis was produced in one of the paired rats by repeated i.p. injection of rat kidney suspensions incorporated in Freund's complete adjuvants. Nephrotic renal disease was noted in all 9 non-injected parabionts, simultaneously with or shortly after onset of proteinuria in the injected rat. In the 5 parabiosed pairs in which the injected rat failed to develop renal disease, the parabiont that had not been treated did not develop nephrotic renal injury. 2. The same antigenic material was given 3-9 times at 2-week intervals by i.v. injection to 12 rats. Slight and inconsistent proteinuria was noted in 5 and histological alterations of glomerular structures were observed in 2. A nephrotic syndrome of comparable severity was not noted in any of these animals. 3. Suspensions of lymphocytes obtained from the spleens of rats with incipient autoimmune renal disease were given by i.v. injection to 9 litter mates in which immune tolerance had been established at birth. Severe membranous glomerulonephritis was produced in 2 of these animals that had been subjected previously to unilateral nephrectomy. None of the 6 identically treated control rats developed renal disease.

1. Heymann, W., Hackel, D. B., Horwood, S., Wilson, S. G. F., Hunter, J. L. P., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 660.

2. Heymann, W., Hunter, J. L. P., Hackel, D. B., *J. Immunol.*, 1962, v88, 135.

3. Hunter, J. L. P., Hackel, D. B., Heymann, W., *ibid.*, 1960, v85, 319.

4. Hess, E. V., Ashworth, C. T., Ziff, M., *J. Exp. Med.*, 1962, v115, 421.

5. Paterson, P. Y., *ibid.*, 1960, v111, 119.

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Responses to Gonadotrophin in Intact and Hypophysectomized Immature Female Rats as Related to Age.* (27858)

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The development of maximal responsiveness to gonadotrophin of the ovary of the immature rat is closely associated with the chronological age of the animal's endocrine system(1). Current interest in the relationship between ovarian reactivity and age has been aroused by recent studies of induction of ovulation in immature mice and rats(2-6), and the possible use of this response as a means of bioassay for ovulatory gonadotrophin(s)(7-9). The use of intact immature animals for assay of gonadotrophins in place of hypophysectomized ones implies that endogenous gonadotrophins are either absent or that their presence has little or no influence upon the organ responses to administered exogenous gonadotrophins. Indirect evidence that the immature animal secretes gonadotrophin has been adduced from bioassays of pituitary content at varying ages prior to puberty and from parabiosis experiments with normal and gonadectomized animals(10-11). The former experiments assume that changes in pituitary content of gonadotrophin can indicate secretion of the hormone (an assumption which many question), whereas the latter experiments apparently do not consider that gonadectomy may effectively alter the normal pattern of development and secretion of the pitui-

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TABLE I. Relation of Age in Response of Normal and Hypophysectomized Immature Female Rats to a Single Dose of Pregnant Mare's Serum Gonadotrophin.

Age in days	No. of animals	Body wt, g ± S.E.	Ovarian wt, mg ± S.E.	Uterine wt, mg ± S.E.	No. of animals	Body wt, g ± S.E.	Ovarian wt, mg ± S.E.	Uterine wt, mg ± S.E.
		Normal controls				Normal injected		
28	9	61.9 ± 2.3	16.4 ± 1.5	30.1 ± 1.6	7	68.3 ± 1.2	28.5 ± 1.1	62.1 ± 7.5
30	15	76.3 ± 2.2	20.3 ± .1	49.2 ± 4.0	14	73.6 ± 1.3	31.7 ± 2.0	86.4 ± 9.8
33	11	90.3 ± 2.5	23.8 ± 2.0	55.8 ± 5.7	13	87.1 ± 2.4	33.4 ± 3.2	104.6 ± 8.9
		Hypox controls				Hypox injected		
28	10	60.5 ± 1.7	9.6 ± 3.7	22.2 ± 1.3	8	60.6 ± 5.1	18.4 ± 6.6	38.6 ± 4.1
30	6	62.2 ± .8	10.3 ± 1.7	22.7 ± 1.7	9	63.9 ± 1.6	18.0 ± 1.2	34.7 ± 3.5
33	6	71.1 ± 1.3	10.3 ± 1.3	24.4 ± 1.6	9	74.6 ± .7	17.7 ± 1.5	34.4 ± 3.8

tary and thereby not be indicative of the unoperated state. In lieu of these considerations the current study was undertaken to determine whether the presence of endogenous gonadotrophin in immature rats of known age can be detected by a comparison of the target organ responses in normal and hypophysectomized animals after administration of a physiological dosage of a standard gonadotrophin preparation. In addition, it was hoped that such data might provide an estimate in terms of the reference standard employed, of the amount of hormone which was released by the animal's pituitary within the experimental time period.

Materials and methods. One hundred and forty-two rats of the Holtzman and Charles River strains were used in this study. Since no strain differences were detected, the data from both strains were combined. One-half of the animals were hypophysectomized by the supplier at 25, 27, and 30 days of age and were received in the laboratory on the second postoperative day. Upon arrival, the animals were housed in wire cages and given Purina Lab Chow and water *ad libitum*. Hypophysectomized animals in addition to the basic ration received 5% glucose in their drinking water. Normal and hypophysectomized animals were divided into 4 groups. One normal and one hypophysectomized group received a single intraperitoneal injection of 0.5 ml containing 10 I.U. of pregnant mare's serum gonadotrophin (PMS) (Equinex)‡ in an aqueous medium 24 hours before autopsy. The remaining normal and hypophysectomized animals served as controls. Animals were killed at 28, 30, and 33 days of age and the ovaries and adrenals were removed, trimmed of fat and weighed. Uteri were excised, dissected from their adnexa, and blotted lightly between filter paper before weighing. Weight of the adrenal glands was used as a criterion for completeness of hypophysectomy. In selected cases, the sella turcica was carefully checked for pituitary remnants with a stereomicroscope. Animals with remnants were discarded.

‡ A gift from Ayerst Laboratories, Inc., New York.

Results. Three days after hypophysectomy, body and adrenal weights were uniformly depressed at the time intervals investigated. A significant difference in ovarian weights was found in normal control rats between 28 and 33 days of age ($P = <0.02$). Administration of gonadotrophin to normal animals resulted in elevated ovarian weights at all ages. However, no statistically significant differences in ovarian weight relative to age within this group were present. The dosage of 10 I.U. of PMS was able to restore ovarian weight of hypophysectomized animals at 28 and 30 days of age to that of their respective uninjected control animals. At 33 days of age, ovarian weights of PMS treated hypophysectomized rats were significantly lower than the 33-day uninjected control ($P = >0.02$).

In normal animals, uterine weights were significantly increased between 28 and 30, and between 28 and 33 days of age ($P = >0.001$). It follows that the amount of estrogen released by the ovary increases appreciably during this time interval. The enhanced weight of the uteri of the normal groups receiving PMS was fairly constant, having an increment of approximately 40 ± 8 mg when compared to the intact controls.

Uteri of hypophysectomized animals receiving PMS at 28 days of age were returned to a value somewhat in excess of the normal weight of the uninjected control; but by 30 and 33 days, more estrogen was being elaborated by the ovary of the uninjected control than could be evoked by PMS administration from the ovary of the hypophysectomized animals, resulting in a uterine weight of the latter significantly lower than the uninjected control ($P = >0.001$).

Discussion. The interval between 25 and 35 days of age is quite critical in the immature rat. During this time there is a decline in ovarian responsiveness to exogenous gonadotrophin as measured by weight increase(1) and by the induced ovulatory reaction(5); concurrently, the anterior pituitary gonadotrophic cells begin to degranulate, which signifies the onset of pituitary regulation of ovarian function(12).

The evidence from this study indicates that in the intact animal the pituitary and the ovary (as a result of pituitary stimulation) are releasing measurable quantities of gonadotrophic and estrogenic hormone respectively, during the time interval under investigation. Using ovulation as an end-point, Rowlands and Williams(13) found that 7-9 days after hypophysectomy, 40 to 50 g rats (the age was not specified) required more gonadotrophin than normal animals, suggesting that the intact animal's pituitary elaborates gonadotrophin. And, very recently, McCormack and Meyer(14) by use of selectively timed barbiturate blockade and hypophysectomy were able to show a reduction in ova induced to ovulate in 24-day-old animals, which they interpreted as indicating a diurnal release of hormone at this early age.

In our study, the differences in ovarian and uterine weight of the normal controls and in the uterine weight of the PMS treated normal rats were statistically significant. The fact that ovarian weight in the treated normal animals did not follow the significant weight gain pattern with advancing age as shown by the uterine responses in the same animals, indicated that weight responses to exogenous gonadotrophin (and presumably endogenous gonadotrophin also) do not reflect the steroid producing capacities of the ovary, a finding similar to that observed in mice by Lamond (15).

Our data show that in hypophysectomized animals to 28 and 30 days of age, 10 I.U. of PMS was able to return the ovarian weight to the level of the intact control. Uterine weights of hypophysectomized animals were restored by the administered gonadotrophin only at 28 days; more gonadotrophic stimulation of estrogen secretion in the ovaries of these animals is necessary at 30 and 33 days to raise the uterine values to that of the uninjected normal control. Thus, the amount of endogenous gonadotrophin circulating in normal animals at 28 days of age may be estimated as approximately equivalent to the activity of 10 I.U. of PMS as a reference standard.

Summary. Ovarian and uterine responses of 28-, 30-, and 33-day-old normal and hypophysectomized rats to a single dosage of pregnant mare's serum (PMS) gonadotrophin 24 hours prior to autopsy indicate that the intact pituitary is elaborating measurable amounts of gonadotrophin during this time interval. The amount of endogenous gonadotrophin circulating in the intact 28-day animal is approximately equivalent to 10 I.U. of PMS as a reference standard. Larger equivalent quantities of gonadotrophin appear to be circulating at 30 and 33 days of age as adjudged by the end organ responses.

1. Price, D., Ortiz, E., *Endocrinology,* 1944, v34, 215.

2. Gates, A. H., Runner, M. N., *Anat. Rec.,* 1957, v128, 554.

3. Lamond, D. R., *J. Endocrinology,* 1960, v20, 277.

4. Ladman, A. J., Runner, M. N., *Proc. 1st Congr. Endocrinol. Periodica, Copenhagen,* 1960, p199.

5. Zarrow, M. X., Wilson, E. D., *Endocrinology,* 1961, v69, 851.

6. Cunningham, F. J., *J. Endocrinol.,* 1962, v24, 215.

7. Runner, M. N., *Anat. Rec.,* 1957, v128, 614.

8. Zarrow, M. X., Caldwell, A. L., Hafez, E. S. E., Pincus, G. *Endocrinology,* 1958, v63, 748.

9. Butt, W. R., Crooke, A. C., Cunningham, F. J., Kovačic, N., *J. Endocrinol.,* 1962, v24, 223.

10. Burrows, H., *Biological Actions of Sex Hor-*

mones, University Press, Cambridge, 2nd Ed., 1949, p40.

11. Greep, R. O., *Sex and Internal Secretions,* Williams & Wilkins Co., Baltimore, Md., 3rd Ed., 1961, v1, p257.

12. Siperstein, E., Nichols, C. W., Jr., Griesbach, W. E., Chaikoff, I. L., *Anat. Rec.,* 1954, v118, 593.

13. Rowlands, I. W., Williams, P. C., *J. Endocrinol.,* 1943, v3, 310.

14. McCormack, C. E., Meyer, R. K., PROC. SOC. EXP. BIOL. AND MED., 1962, v110, 343.

15. Lamond, D. R., *J. Endocrinol.,* 1958, v17, 218.

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Effect of Adrenalectomy and Adreno-Demedullation on Experimental Edema as Measured under Repeated Ether Anesthetizations.* (27859)

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Evidence has been reviewed which shows that various forms of stress cause an increase in adrenal activity with a subsequent release of corticosteroids and catecholamines(1). Since anesthesia with ethyl ether releases epinephrine(2), our experiments were performed to determine the effect of ether on experimental, mustard-induced, hind limb edema formation in adrenalectomized, adreno-demedullated and intact rats. The studies were designed to shed light on the role played by the medullary and cortical portions of the adrenal gland in the general process of inflammatory edema.

Method. Male Sprague-Dawley rats (Charles River Breeding Laboratories–CD), 150-220 g, were used in all studies. Adrenalectomies were performed bilaterally the day prior to the experiment and the rats were allowed 0.9% saline *ad libitum* thereafter. Adrenal demedullations were done bilaterally about 30 days before the animals were used (3). Ethyl ether (U.S.P.) was used for the surgery and all limb volume determinations conducted on anesthetized animals.

Limb volumes were determined by volumetric displacement according to a previously described method(4,5) adapted for our use. The foot, up to the hair line, was placed in a tube of mercury and the volume, in milliliters, determined by the amount of mercury displaced. The edema was induced by the subplantar injection of 0.1 ml of an irritating 2% aqueous mustard† suspension(6) into the left hind limb. The injections were given immediately after the initial limb volume reading. The amount of edema present was calculated by subtracting the initial limb volume of each animal from the subsequent volumes recorded at different time intervals after injection of the irritant. Limb volumes were determined before and from 1 to 5 times after the mustard injection. The time schedule shown in Table I was followed for intact, adrenalectomized and adreno-demedullated animals given ether for each determination as well as for intact rats in which limb volumes were determined without anesthesia. All presented data are the result of 3 experiments done on different days with 5 to 8 rats per group.

TABLE I. Schedule of Limb Volume Determinations.

No. of limb volume determinations after initial determination	Time of determinations (hr after mustard inj)
1	6
2	2, 6
3	1, 2, 6
4	1, 2, 4, 6
5	.5, 1, 2, 4, 6

Results. Table II shows the amount of

* We thank Mrs. Christina Lockwood for assisting in limb volume determinations.

† Colman's Mustard (double superfine) distributed by R. T. French Co., Rochester, N. Y. Mustard used in these experiments was from a single lot well mixed to assure uniformity of irritancy.