phrotic syndrome makes it unlikely that the failure of liver, lung and muscle to produce the same effect was due to lower concentration of tissue proteins in these suspensions. Further immunological studies to evaluate this view are in progress.

Summary. 1) A severe nephrotic syndrome was noted in 20 rats given Freund's adjuvants and a supernate of rat kidney suspension by intraperitoneal injection. When kidney tissue was replaced by liver, only 3 of 21 animals developed a much milder renal disease. Lung or muscle suspensions failed to induce proteinuria in 10 rats. 2) In large doses (0.5 ml) Freund's adjuvants alone produced a mild nephrotic disease in 3 of 10 rats. Even though the smaller amounts of the adjuvants usually used (0.25 ml) never produced proteinuria, slight histological alterations of the glomerular structure were frequently noted. 3) In contradistinction to other tissue suspensions addition of rat kidney supernate enhanced the effect of adjuvants markedly and regularly. 4) The small amounts of rat kidney proteins used, support the interpretation that an iso- and autosensitization mechanism is at play.

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Precocious Sexual Development in Female Rats with Hypothalamic Lesions.* (24737)

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Appropriate hypothalamic lesions may impair selectively the secretion of several anterior pituitary hormones(1,2). This strongly suggests that the nervous system can exert discrete stimulating, or at least permissive, influences upon anterior lobe function. The converse possibility, that it may also act to suppress trophic hormone secretion, has been raised by several recent findings(3-6). This report, on effects of hypothalamic lesions in weanling male and female rats, deals further with this possibility.

Methods. Holtzman rats were weaned and subjected to bilateral electrolytic hypothalamic lesion placement at 18-19 days of age. Littermates of the same sex and approximate weight served as sham- and unoperated controls. Each litter was kept in a small cage and fed Big Red dog pellets and tap water, *ad lib.* During the first 3-4 post-operative days they were also given evaporated milk. Litters were sacrificed 10-15 days after surgery *i.e.,* at 28-33 days of age. Brains were fixed in 10% neutral formalin, trimmed to blocks of diencephalon, Tissuemat-embedded and sectioned serially at 10 μ. Every 9th and 10th section was stained with thionine for lesion localization studies. Other tissues were fixed, after rapid trimming and weighing, in Susa-picric acid or Helley's fluid. Routine 6 μ sections were stained with Gomori's trichrome-hematoxylin. Ovaries, sectioned serially at 10 μ, were also trichrome-stained. Hypophyses of females were examined by means of both periodic acid - Schiff reagent - hematoxylin (PAS - 6 μ) and aldehyde-fuchsin - trichrome - hematoxylin (AF - 4 μ) stained

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TABLE I. Body and Organ Weights of Immature Female Rats with Hypothalamic Lesions, and of Littermate Controls.

Age (days)	Group*	Body wt, g	Uterus, mg	Ovaries, mg	Adrenals, mg
28	Cont. (2)	77	44	22	18
	H Les. (2)	54	110	14	15
30	Cont. (8)	81 ± 2.6†	47 ± 3.2	21 ± 1.3	21 ± .7
	A Les. (1)	63	29	14	11
	0 " (4)	74 ± 3.5	47 ± 3.5	20 ± 1.2	19 ± 5.3
	H " (5)	75 ± 2.3	135 ± 29	21 ± 1.1	21 ± 1.0
31–32	Cont. (5)	93 ± 3.1	43 ± 1.8	21 ± 1.4	18 ± 1.1
	0 Les. (1)	84	47	19	16
	± " (3)	72	68	19	17
	H " (3)	79 ± 2.7	179 ± 22	21 ± 1.6	19 ± .8
33	Cont. (8)	98 ± 2.9	61 ± 5.0	23 ± .8	23 ± .3
	A Les. (1)	62	40	11	12.5
	H " (9)	73 ± 2.1	134 ± 10.7	26 ± 1.8	21 ± 3.0

* Cont. = sham and/or unoperated littermates; H Les. = lesions resulting in uterine hypertrophy; A Les. = lesions producing gonad atrophy; 0 Les. = lesions with no effect on gonads; ± Les. = lesions with slight or dubious effects. Figures in parentheses indicate No. of rats in group.

† Stand. error of mean.

sections. Only representative samples of testis, seminal vesicle, prostate, adrenal, uterus and vagina were inspected microscopically, since organ size was felt to be an adequate index of functional state. Actual organ weights have been used since experimental rats grew less than sham controls and it would be misleading to express the figures on body weight basis.

Results. Reproductive system. Males. There was no hypertrophy of testis, seminal vesicle or prostate gland in any of 36 male rats, with lesions virtually identical to those in the females. Since similar lesions frequently result in marked accessory sex gland hypertrophy in adult males(7), this was somewhat surprising. Eleven males showed gonad atrophy. Their lesions destroyed most of the arcuate nucleus (Fig. 11).

Females. Uterine weight (minus fluid content) averaged 108 ± 10.4 mg for 29 females with lesions (including 2 with uterine, ovarian and adrenal atrophy and 8 without marked uterine hypertrophy). Uterine weight averaged 50.5 ± 2.7 mg for 23 littermate controls. Since controls exhibited some uterine and ovarian growth between 28 and 33 days of age, the data have been grouped by age as well as by uterine weight in the Table.

All enlarged uteri had proliferative endometria but showed no signs of progestational activity. Vaginal smears could not be followed because in many cases the closing membranes were still intact at sacrifice, although they could then be ruptured with very slight perineal pressure. Some vaginas were studied microscopically. A few showed marked cornification (Fig. 2), in contrast to controls (Fig. 1). Full vaginal cornication was found in rats with 4-5 fold enlargement of the uterus. Rats with less (3-fold) uterine enlargement exhibited only vaginal mucification and/or slight cornification.

Control ovaries weighed between 16.8 and 26.8 mg and were devoid of large follicles (Fig. 3). Ovaries of rats with uterine hypertrophy weighed between 11.5 and 33.7 mg. Those which weighed less than controls contained only one (Fig. 4) or a few (Fig. 5) enlarged follicles, which appeared to have developed at the expense of the rest of the gland. Ovaries of 7 rats (uterine weights 94.2 to 186.3 mg) weighed 28.7 ± 1.3 mg and contained corpora lutea (Fig. 6). These corpora lutea appeared to be new and, in a given ovary, all of the same age. Some of them were quite small, and none were over 800 μ in diameter. In several, trapped ova (Fig. 7), or cysts suggesting degenerated entrapped ova, were seen. Similar structures, called corpora lutea atretica (CLA), have been found in immature rats treated with large doses of luteinizing preparations(8).

Male hypophyses were not examined micro-

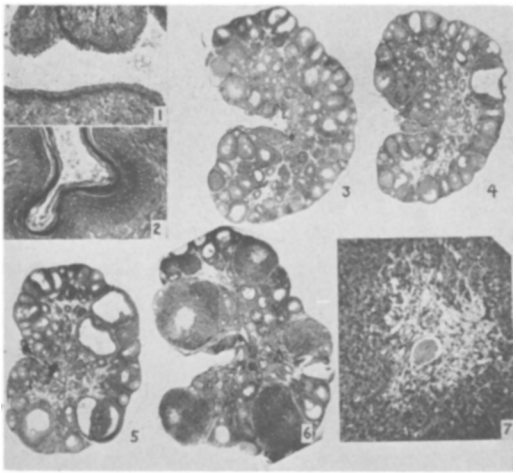

FIG. 1. Vagina of 31-day-old control with 23 mg ovary and 44 mg uterus. × 152.
FIG. 2. Vagina of 32-day-old lesion-bearing rat with 18 mg ovary and 207 mg uterus. × 152.
FIG. 3. Control ovary, wt 21.5 mg, no large follicles. Uterine wt 44 mg. × 18.
FIG. 4. Experimental ovary with one large follicle. Ovarian wt 17 mg. Uterine wt 107 mg. × 18.
FIG. 5. Experimental ovary with several large follicles. Ovarian wt 19 mg. Uterine wt 214 mg. × 18.
FIG. 6. Experimental ovary with several corpora lutea. Ovarian wt 34 mg. Uterine wt 128 mg. × 18.
FIG. 7. Ovum entrapped in luteinized follicle. × 135.

scopically. Those of female controls were not rich in PAS-positive gonadotrophs. Lesions definitely did not increase size, number or staining intensity of these cells and may even have decreased them in some cases. Nevertheless, some gonadotrophs were distinguishable in every gland. No differences in AF staining β-cells were seen. Acidophiles were sparse in some experimental glands, but this could not be correlated with uterine size.

The only adrenal effect noted in either sex was atrophy. It was not seen frequently.

Lesion localization. Because of the softness of skulls of 18-19-day-old rats, there was considerable variation in stereotaxic lesion placement. (Figs. 8-10, 12). Lesions which produced CLA resembled either that shown in Fig. 9 (and 12a) or the one depicted in Fig. 10 (and 12b). Such lesions apparently damaged the arcuate nuclei only partially, sparing portions. The 2 females and 11 males with gonad atrophy had lesions which damaged the arcuate nuclei more than those shown in Figs. 9 and 10.

It is clear that uterine enlargement and formation of CLA were brought about by lesions which did not involve the anterior hypothalamus (Fig. 9). On the other hand, uterine enlargement (without ovarian luteinization) also resulted from strictly anterior hypothalamic lesions (Fig. 8).

Discussion. Two varieties of precocious sexual development were found in our female rats. Strictly anterior hypothalamic lesions (Fig. 12a) resulted in premature follicular growth and uterine hypertrophy. Rats with more caudal lesions, which destroyed part of the arcuate nucleus but left an adjacent portion intact (Fig. 12b), also exhibited uterine hypertrophy but their ovaries contained corpora lutea. The 2 types of gonadal hypertrophy were distinguishable only on the bases of ovarian structure and lesion location.

In the first (follicular) variety, some LH must have been secreted for estrogen secretion to occur(9). However, such a low level of LH secretion might well have been present in the controls. In any event, the effect seems to reflect mainly increased FSH secretion.

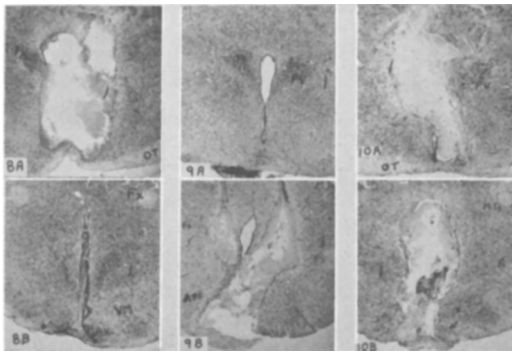

FIG. 8. Coronal sections through hypothalamus of rat with an anterior lesion. Ovarian wt 19 mg, follicular growth but no luteinization. Uterine wt 129 mg. A. Section just behind optic chiasm. B. Section at level of median eminence. For symbols, see legend to Fig. 11. × 20.
FIG. 9. Coronal section of lesion which spared anterior hypothalamus but involved arcuate nuclei. Ovary weighed 33 mg and contained several corpora lutea. Uterus weighed 127 mg. Sections A and B through levels comparable to Fig. 8, A and B. × 20.
FIG. 10. Sections through lesion damaging both anterior hypothalamus and arcuate nucleus region. Ovary, shown in Fig. 6, weighed 34 mg. Uterine wt 128 mg. A. Section through anterior part of lesion. B. Section through arcuate nucleus region. Note intact arcuate nucleus on one side (arrow). × 20.

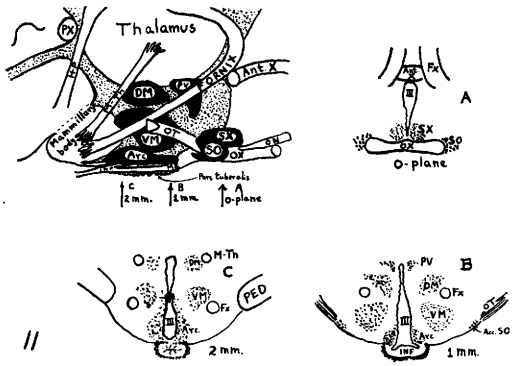

FIG. 11. Scheme of rat hypothalamus. Upper left: sagittal section through third ventricle (stippled), with nuclei and fiber tracts lateral to midline superimposed by projection. A, B and C: transverse sections at levels indicated by arrows below sagittal section. Symbols: ON, OX, OT—optic nerve, chiasm, and tract. Nuclei: SX—suprachiasmatic, SO—supraoptic, Acc. SO—accessory supraoptic, PV—paraventricular, DM—dorsomedian, VM—ventromedian, and Arc.—arcuate, respectively. FX—fornix; M-Th and H-P—mammilothalamic and habenulopeduncular tracts; AX and PX—anterior and posterior commissures.

The second (luteal) variety of precocious ovarian function was characterized by small corpora lutea. Although this suggests that LH was the chief excitor of gonadal activity, FSH must also have been secreted for follicular growth to have occurred at all. In a normal cycle, not much LH is secreted during the follicular growth phase. It is possible that if both FSH and LH are secreted simultaneously, luteinization may transpire before FSH has had time to produce optimal follicular growth. In any event, both FSH and LH secretion appear to have been increased, perhaps equally or perhaps with LH predominating. More detailed analysis of these findings must await further knowledge of the secretion and effects of gonadotrophins in rats of this age.

Can either or both of these findings properly be termed "precocious puberty"? The fact that most experimental females exhibited signs of estrogen secretion at time of sacrifice strongly suggests that they were not undergoing normal sexual cycles. These rats were killed (to avoid spontaneous estrus in the controls) before any vaginal smear study of cycles would have been possible. Before applying the term "puberty" to the findings, it seems desirable to follow such rats closely for a longer time to see if a) premature sexual development is maintained, b) premature, but otherwise normal, sexual cycles are present and c) precocious reproduction is possible.

Whether the heightened gonadotrophin secretion consisted only of increased hormone release or also of augmented elaboration and storage, has not been answered satisfactorily by this experiment. Although pituitary structure suggested that gonadotrophin stores were decreased slightly in experimental rats, this question must await application of appropriate bioassay methods.

There are two possible explanations for the increased hypothalamic-pituitary activity found. Either the lesions destroyed suppressor mechanisms or they activated stimulating ones. Both explanations may be invoked in interpreting the varied results of this study. The postulate(3-6) that there is an FSH inhibiting system in the anterior hypothalamus would not be in disaccord with the present findings of increased FSH secretion after an-

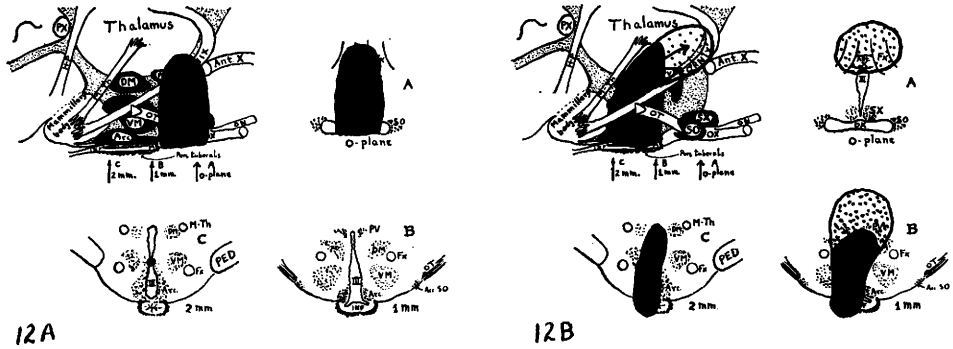

FIG. 12. A. Lesion (solid black, superimposed on Fig. 11) of the type shown in Fig. 8, did not result in ovarian luteinization. B. Lesions (solid black, or solid black plus stippling, superimposed on Fig. 11) of the type depicted in Fig. 9, did result in ovarian luteinization.

terior lesions, *if it be assumed* (not unreasonably) *that some LH is normally secreted by rats of this age.* Whether there may also be an anterior hypothalamic LH suppressor mechanism is less certain (see below). No conclusive evidence for such an assumption evolved from our study.

The heightened LH (and FSH?) secretion in rats with incomplete arcuate nucleus lesions might, of course, indicate an LH suppressing system in, or acting on, this part of the median eminence. However, it seems at least equally likely that some residual neurones within a hyperemic zone at the periphery of these lesions may have been stimulated. In this and other(1,10,11) studies, a greater degree of destruction of the arcuate nucleus region almost invariably resulted in marked gonad atrophy.

If neurons in the arcuate nucleus region normally stimulate both FSH and LH secretion, the possibilities that their functions might be increased either by removing suppressor mechanisms or by irritating these cells directly (as by including them in an inflammatory zone) must both be considered. Removal of a specific suppressor might be expected to bring about a selective secretory increase, whereas irritation would probably have less discrete effects. The follicular type of precocious sexual development may well have been the result of disrupting a specific FSH suppressor system. Correct interpretation of the luteal type must await further study.

Detailed review of what is known about hypothalamic gonadotrophin-regulating mechanisms is beyond the scope of this paper, but it may be valuable to summarize here a few important points: a) Fairly complete destruction of the arcuate nucleus region, regardless of other hypothalamic damage, leads to gonad atrophy(1,10,11). Anterior hypothalamic lesions b) produce precocious sexual development, c) may produce prostate and seminal vesicle hypertrophy(7) and d) in females, block the effects of estrogen on gonadotrophin secretion(5, also Bogdanove and Crabill—unpublished). e) Smaller, more lateral anterior lesions produce constant estrus and apparent impairment of LH secretion(9) which may be relieved by progesterone administration(12). f) Ovarian autografts in the anterior hypothalamus decrease uterine size(6). Points b, d and f suggest an FSH inhibitory mechanism, point c, an LH inhibitor. Point e suggests that another anterior mechanism, located close to the preceding, is essential for the LH discharge which precipitates ovulation. Interpretation of the effects of progesterone in rats with constant estrus is obscure. Because of point a, it seems necessary to suppose that the gonadotrophin suppressing and LH triggering mechanisms act *via* the stimulating systems in the arcuate nucleus region, and not directly on the adenohypophysis.

It is hard to explain why virtually identical lesions produced such dramatic effects in immature females, but had no effect in immature males. Perhaps there was, in both sexes, a very slight increase of gonadotrophin release, adequate to stimulate the ovary but insufficient to affect the testis. On the other hand, the hypothalamic-pituitary gonadotrophin system may be more mature in females of this age than in males.

Summary. Two types of precocious sexual development were found in female rats with hypothalamic lesions. Anterior lesions were followed by precocious ovarian follicular growth with uterine and vaginal manifestations of estrogen secretion. Lesions damaging part of the arcuate nucleus had not only these sequelae, but also ovarian luteinization. These results are interpreted to mean that anterior lesions increased FSH release, whereas arcuate nucleus lesions increased both FSH and LH release. Together with results of other studies, they suggest that anterior lesions may damage an FSH inhibitory mechanism. The results of arcuate nucleus lesions do not necessarily imply that an LH inhibitory mechanism was destroyed, however. The possibility that such lesions may act by irritating a gonadotrophin stimulating mechanism is discussed.

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Site of Fatty Acid Absorption.*† (24738)

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It has been recently reported from this laboratory(1) that segments of the hamster intestine are capable of absorbing fatty acids from the mucosal side of an *in vitro* preparation. The fatty acids appeared in the serosal solution predominantly in the form of esterified fatty acids. The serosal esterified fatty acids were 90% or more as triglyceride.‡ During the investigation, it was recognized that sacs, prepared from the same animal, exhibited a variation in absorptive activity depending upon the portion of the small intestine employed. Location of fat absorption in the small intestine of different species has been reported in a number of investigations as reviewed by Turner(2). These investigations were primarily concerned with determination of the fat content of sections of intestine following ingestion of triglycerides. Because of the lipolysis which occurs in the intestine, it is difficult to interpret these results with regard to absorption of the specific products of the hydrolysis of ingested triglycerides. In an attempt to determine whether fatty acids, as the sole lipid placed on the mucosal side, are absorbed and transported to a greater degree in certain areas of the isolated small intestine, the present investigation was undertaken.

Materials and methods. A solution of C^{14}-palmitic acid-albumin complex containing 200 mg % glucose was prepared as previously described(1). Small everted sacs were made from successive segments of the hamster intestine from upper jejunum to iliocecal junction according to a modified procedure(1) of Wilson and Wiseman(3). The sacs were filled with approximately 0.5 ml of bicarbonate buffer, pH 7.4, containing 200 mg % glucose. Intestinal segments were incubated for 2½ hours at 37°C in 4 ml of the fatty acid-albumin complex in an atmosphere of 95% O_2 and 5% CO_2. Aliquots of the serosal and mucosal solutions as well as from the homogenized intestinal wall were plated for counting(1). The lipids were fractionated into free and esterified fatty acids according to the procedure of Borgström(4).

Results. The results from successive segments of intestine from upper jejunum (sac 1) to iliocecal junction (sac 5) are given in Table I. Averages of similar segments of 3 animals are shown as the total activity recovered from mucosal and serosal solutions and intestinal wall. Although there was variation in ability to absorb fatty acid from animal to animal, each of the individual experiments exhibited decreasing serosal and intestinal wall activities in the lower portions of the small intestine. The lowered activity was confirmed by similar decrease in fatty acid uptake from the mucosal solution. The combined lipids from the mucosal solutions and intestinal walls of similar sacs were fractionated into glycerides and fatty acids. The corresponding serosal solutions from sacs 1 and 2 in the 3 experi-

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