TABLE II. Influence of Hypophysectomy and of Estradiol on Phosphorus Content of the Uterus.

Group A—Untreated	
Adult intact	$y = .0011x$
" hypoph.	$y = .0017x$
Immature intact	$y = .0018x$
" hypoph.	$y = .0024x$
Group B—Estradiol treated	
Adult intact + Est.	$y = .0012x$
" hypoph. + Est.	$y = .0016x$
Immature hypoph. + Est.	$y = .0015x$
" intact + Est.	$y = .0015x$

over in the uterus and overcomes the retarding influence of hypophysectomy on this target organ. It acts as an "equalizer" with respect to the phosphorus in the uterus of the immature intact, immature hypophysectomized, and adult hypophysectomized rat.

Investigations conducted in this laboratory showed the total phosphorus in the liver in both the immature and adult rat(4) to be the same per unit weight of tissue. The reason that the uterus does not act in this manner in the immature untreated rat as compared to the uterus of the adult rat may be explained by the fact that the uterus is a target organ, subject to changing metabolic processes with age. As maturity is approached the ovarian hormones, such as estradiol, may stimulate the phosphorus turnover and bring about an altered phosphorus content in the adult rat uterus.

From these observations it would appear justifiable to postulate that ovarian hormones, produced by way of pituitary activity, are important factors responsible for gradations of the phosphorus content of the uterus in varying conditions of age and glandular activity.

Conclusions. 1. The phosphorus content reaches a maximum per mg of uterine tissue in the immature untreated hypophysectomized animal. 2. The phosphorus content per mg uterine tissue is lowest in the adult and adolescent group in both the untreated and estradiol treated rats. 3. The phosphorus content per mg uterine tissue of estradiol treated animals is less than in the untreated animals. 4. Estradiol increases uterine weight and presumably stimulates phosphorus turnover but it does not increase the phosphorus content of the uterus. 5. The importance of estrogenic and pituitary hormones with respect to phosphorus metabolism in the uterus of the rat has been emphasized.

4. Strickler, Herbert S., Cutuly, Eugene, Grauer, Robert C., and Wolken, Jerome J.; Unpublished data.

Received May 31, 1951. P.S.E.B.M., 1951, v77.

Evidence of Hypothalamic Control of the Pituitary Release of Thyrotrophin.* (18862)

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Although it has been postulated for many years that the hypothalamus in some manner regulates the secretion of thyrotrophin by the hypophysis, clear-cut experimental evidence demonstrating this phenomenon has not yet been presented(1). The present investigation was designed to provide a maximal stimulus for thyrotrophin release by feeding animals with hypothalamic lesions a diet containing propylthiouracil. In addition to thyroid size and histologic evidence of activity the iodide-concentrating capacity of the thyroid glands was determined, since this had previously been shown to be the most sensitive index of endo-

* This work was aided in part by grants to Dr. E. B. Astwood from the American Cyanamid Co. and the U. S. Public Health Service.

† U. S. Public Health Service Postdoctoral Research Fellow of the National Cancer Institute.

1. Harris, G. W., *Physiol. Rev.*, 1950, v28, 139.

TABLE I. Data of Rats with Hypothalamic Lesions. T/S I^{131} ratio refers to the concentration of I^{131} in comparable quantities of thyroid tissue and serum.

Rat	Initial body wt	Final body wt	Thyroid wt, mg	Thyroid wt, mg/100 g body wt	T/S I^{131} ratio	Pituitary damage	Thyroid hyperplasia	Hypothalamic lesions
CR-1	251	277	14.3	5.2	22.3	Severe	Absent	Paraventricular to middle of ventromedian n. Extends through ventral surface hypothalamus bilaterally and more extensive rt. side.
CR-2	256	279	13.7	4.9	128	None	"	Suprachiasmatic to middle of ventromedian n. Extends across midline, more extensive on rt.
CR-3	239	256	14.1	5.5	247	"	"	Paraventricular to middle of ventromedian n.
CR-4	223	224	10.4	4.6	81	Small infarct left lobe	"	Middle to caudal end of ventromedian n.
CR-5	221	234	10.75	4.6	258	None	"	Beginning of paraventricular to ventromedian n. Extends across midline.
CR-6	258	249	16.4	6.6	215	"	"	Lesion very small, extending across midline at ventral surface of hypothalamus just behind suprachiasmatic n.
CR-7	255	223	19.1	8.6	433	"	Moderate	Paraventricular to ant. half ventromedian n. Lesions displaced to left.
CR-8	258	258	13.5	5.2	147	"	Absent	Paraventricular to anterior ventromedian n. Extends through surface of hypothalamus on left, 1 mm from surface on rt.
CR-9	246	252	20.15	8	395	"	"	Suprachiasmatic to anterior paraventricular n.

genous thyrotrophin activity available(2,3).

Materials and methods. Male and female Sprague-Dawley rats of the Harvard and Charles River strains were used. Hypothalamic lesions were produced in animals weighing 230-300 g by a modified Krieg stereotaxic instrument(4). Lesions were placed from 6 to 7 mm anterior to the ear plugs and 1 mm on each side of the midline. A varnished steel needle or copper wire with only the tip bare was used as the localizing electrode. After insertion through burr-holes in the dorsum of the skull, this was lowered until it encountered the base of the skull, when it was raised a distance of 0.5-1.0 mm. A direct current of 2.5-8.0 ma was applied for 15-30 seconds on each side. Ether was found to be a more satisfactory anesthetic than barbiturates.

After allowing one to 3 weeks for recovery from the operation to take place, the animals were given a diet of 0.03% propylthiouracil in mink chow and killed at the end of 10 days. The thyroids were dissected and weighed. The head was then removed and all excess tissue including the bony dorsum of the skull trimmed away, leaving the brain and base of the skull with the pituitary intact. All tissues were fixed in 10% formalin. After several days of fixing, 10 μ serial sections were made of the hypothalamic area, every tenth section

2. VanderLaan, J. W., and VanderLaan, W. P., *Endocrinology,* 1947, v40, 403.

3. VanderLaan, W. P., and Greer, M. A., *Endocrinology,* 1950, v47, 36.

4. Krieg, W. J. S., *Quart. Bull. Northwestern Univ. Med. School,* 1946, v20, 199.

TABLE II. Data of Control Rats.

Rat	Final body wt	Thyroid wt, mg	Thyroid wt, mg/100 g body wt	T/S I^{131} ratio
1	296	41.4	14	456
2	290	34.15	11.8	298
3	278	29	10.4	232
4	286	44.1	15.4	283
5	307	50.9	16.6	242
6	260	28.2	10.9	285
7	315	35.3	11.2	261
8	296	34.75	11.7	270
9	262	25.2	9.6	283
10	275	35.8	13	343
11	251	31.2	12.4	239
12	271	44.55	16.4	290

being mounted. The pituitary and thyroid were sectioned separately, coronal pituitary sections at 10 μ being used in order to visualize both lateral lobes of the pars anterior adequately. The brain sections were stained with 1% toluidine blue for 6 hours while pituitary and thyroid were stained with hematoxylin and eosin. When determining the iodide-concentrating ability of the thyroid, one lobe was used for determination of radioactivity, the other being fixed in formalin.

The iodide-concentrating capacity of the thyroid was determined as previously described(3). Ten mg of propylthiouracil were injected subcutaneously 30-60 minutes before the injection of a 5 μc tracer dose of I^{131}. One to 2 hours later the animals were killed and the concentration of I^{131} per unit of thyroid tissues compared to the concentration in an equivalent amount of serum. This I^{131} was present only as inorganic iodide since the previous injection of propylthiouracil had effectively blocked all protein binding of iodine.

Results. Preliminary experiments in 19 rats had indicated that lesions in the rostral and tuberal mid-hypothalamic areas were most likely to give positive results. Accordingly, an attempt was made to place the lesions within the area bounded by the optic stalk and the infundibulum. Eleven animals were operated upon, of which 2 died a few days postoperatively. Twelve control animals of approximately the same weight were treated similarly except for the production of hypothalamic lesions. The results are summarized in Tables I and II.

It can be seen that a marked difference existed in the thyroid response to thiouracil feeding between the intact and operated animals. Whereas the thyroid weights of the control animals varied from 9.6 to 16.6 mg per 100 g body weight, averaging 12.8 mg, those of the hypothalamic animals ranged from 4.6 to 8.6, averaging 5.9 mg. The thyroids of the operated animals thus apparently did not undergo any appreciable hypertrophy with thiouracil administration, averaging less than half the size of the controls. In fact, they would seem to be even slightly smaller than the thyroids of untreated, intact rats in this laboratory, which usually weigh 7-8 mg per 100 g body weight.

Microscopic examination of the thyroid tissue revealed an atrophic, flat to low cuboidal epithelium in all operated animals except CR-7. In this one instance there was a moderate hyperplasia, although not nearly as intense as in the unoperated animals. The thyroids of the control group, on the other hand, all showed the usual marked hyperplastic changes seen in thiouracil-treated animals.

Perhaps the most surprising finding of the investigation was the marked increase in the iodide-concentrating capacity of the thyroid tissue in the absence of any anatomic evidence of hyperfunction. The thyroid:serum iodide ratio was well above the normal value of 25:1 in all but one animal and in most instances was fully as high as in the control thiouracil-treated animals. The one exception, CR-1, had a large infarct affecting both lateral lobes

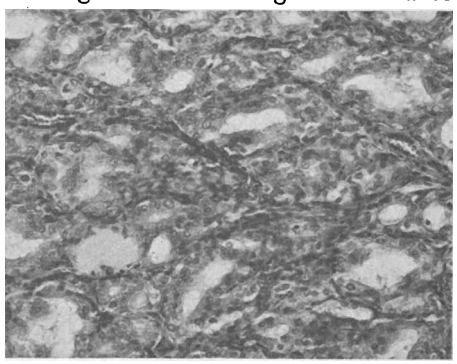

FIG. 1. Typical example of thyroid hyperplasia seen in control rats after 10 days of thiouracil feeding. Notice marked increase in cell height and diminution of colloid. H & E, × 160.

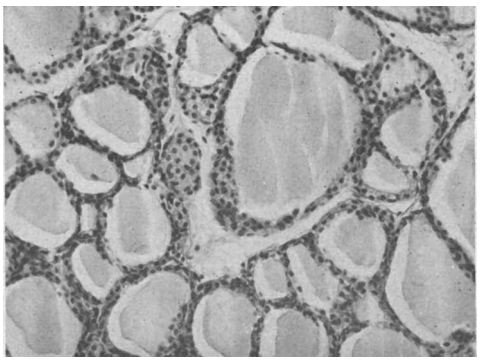

FIG. 2. Thyroid of CR-2. Atrophic appearance with large, colloid-filled follicles. Note flat, atrophic epithelium. H & E, × 160.

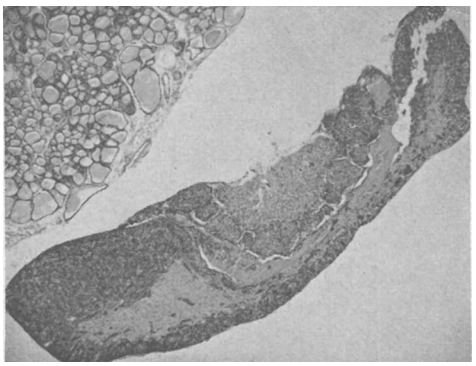

FIG. 3. Coronal section of pituitary of CR-1 showing large infarct affecting both lobes of pars anterior. H & E, × 26.

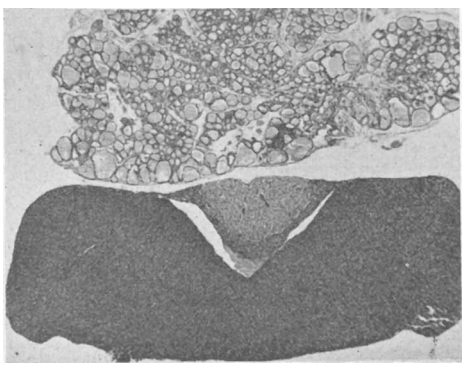

FIG. 4. Coronal section of pituitary and thyroid of CR-8 showing intact pituitary and atrophic thyroid. H & E, × 12.

of the anterior hypophysis, but nevertheless maintained a normal thyroid iodide concentration.

The pituitary glands appeared perfectly normal in all but 2 animals. CR-1 had a large infarct destroying most of both lateral lobes of the anterior hypophysis, as previously mentioned, and CR-4 had a small infarct in the left lobe of the pars anterior.

The hypothalamic lesions were of small to moderate size and were all located in approximately the area intended. The most anterior lesions were just behind the suprachiasmatic nucleus and the most posterior extended to the caudal portion of the ventromedian nucleus. The lesions in CR-1 with the large pituitary infarct extended through the ventral surface of the hypothalamus from the paraventricular to the middle of the ventromedian nuclei and that on the right side was more extensive. The largest lesions were in CR-2 and extended

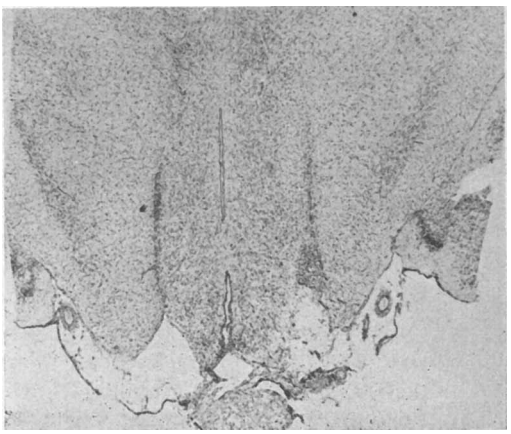

FIG. 5. Coronal section through hypothalamus of CR-4 illustrating bilateral lesions through caudal part of ventromedian nuclei. The infundibular stalk is in the lower midline. H & E, ×10.

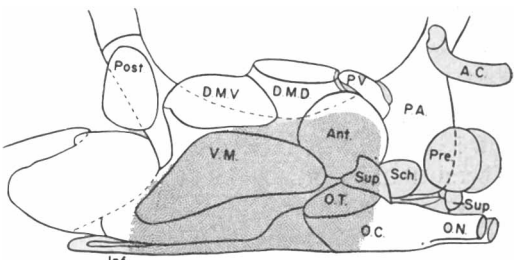

FIG. 6. Scheme of hypothalamus, modified from Krieg. Area of lesions indicated by stippling. A.C., anterior commissure; Ant., nucleus hypothalamicus anterior; D.M.V., D.M.D., ventral and dorsal portions of dorsomedian hypothalamic nucleus. Inf., infundibulum; O.C., optic chiasm; O.N., optic nerves; O.T., optic tract; P.A., nucleus hypothalamicus periventricularis, anterior region; P.V., paraventricular nucleus; Pre., preoptic nucleus; Post., posterior hypothalamic nucleus.

from the suprachiasmatic to the middle of the ventromedian nuclei. The lesions in this animal fused in the mid-line in the region of the anterior portion of the ventromedian nucleus, but did not extend through the ventral surface of the hypothalamus. The smallest lesion was in CR-6, extending across the ventral hypothalamic surface through the mid-line, just behind the suprachiasmatic nucleus. In CR-7, the only animal to show any evidence of hyperplasia of the thyroid epithelium, there was a displacement of the lesions to the left so that they were not bilaterally symmetrical. This was the only animal in which symmetrical lesions were not placed.

Discussion. It is evident that the animals with hypothalamic lesions did not show the usual goitrogenic response to propylthiouracil administration. Since the pituitaries of all but two animals were completely intact and only that of CR-1 showed severe damage, it is extremely unlikely that the absence of thyroid hypertrophy was due to pituitary injury. All animals had a final body weight equal to or greater than that at the time of operation. The one exception was CR-7, whose weight fell from 255 to 223 g. As described above, this was the only animal which showed moderate hyperplastic changes in the thyroid. The weight gain, although not marked, may in part have been due to lesions in the region of the ventromedian nucleus, since Brobeck(5) has established that damage to this area produces obesity.

It is difficult to explain the marked dichotomy between thyroid size and iodide-concentrating ability. An intact pituitary has been shown to be essential for both the goitrogenic response(6) and the increase in thyroid iodide concentration(3) following thiouracil administration. In this investigation it is evident that the usual increase in iodide concentration has occurred without concomitant anatomical evidence of hyperplasia. There has been some previous experimental evidence that these two features may be distinct in that the thyroid: serum iodide ratio falls much more rapidly following hypophysectomy in rats chronically treated with propylthiouracil than does thyroid size or cell height(3).

It seems reasonable to postulate that the hypothalamic lesions in some manner prevented the usual augmentation of thyrotrophin secretion in thiouracil-treated rats. Whether this was qualitative, quantitative, or both is not clear from the data available. It does seem evident that some pituitary thyrotrophin was being formed, since there was a marked increase in thyroid iodide concentration which would not occur in the absence of the hypophysis. Two possibilities might be suggested: (1) The pituitary is unable to secrete enough thyrotrophin to stimulate cellular hyperplasia of the thyroid but is able to secrete the smaller amount needed to increase the thyroid iodide concentration; (2) Two different thyrotrophins are secreted by the pituitary, one causing cellular hyperplasia and the other an increase in thyroid function. In the presence of hypothalamic lesions similar to those described above, the pituitary is able to secrete only the latter hormone. With the presently available evidence, it is impossible to state which, if either, of these theories is correct. Even the animal with severe pituitary damage was able to maintain a normal thyroid iodide concentration, so that in none of the animals was hypophyseal function completely absent. Normal animals fed a thiouracil diet for a comparable time immediately following hypophysectomy almost invariably have a thyroid:serum ratio below 10:1 and frequently closely approaching 1:1(7).

These experiments do not determine the nature of the hypothalamic influence on the pituitary. This may be either neural or humoral, but since most investigators have found only a very scanty nerve supply to the pars anterior(1) and since it is established that the hypophyseal portal system carries blood to the pituitary down the hypophyseal stalk(8), it would seem more probable that the influence might be humoral. Whether the hypothalamic area damaged exerts a primary influence on

5. Brobeck, J. R., *Physiol. Rev.*, 1946, v26, 541.

6. Astwood, E. B., Sullivan, J., Bissell, A., and Tyslowitz, R., *Endocrinology*, 1943, v32, 210.

7. Greer, M. A., *Endocrinology*, 1949, v45, 178.

8. Barrnett, R. J., and Greep, R. O., *Science*, 1951, v113, 185.

the pituitary or only transmits tracts from other centers is also not clear.

The results of certain investigators with pituitary stalk-sectioned rats is compatible with the data obtained in this investigation. Brolin(9) found that the usual marked hypertrophy and hyperplasia of pituitary basophiles following thyroidectomy did not occur in stalk-sectioned rats and Greep and Barrnett observed a markedly decreased goitrogenic response following 2-3 weeks of thiouracil feeding in intact, stalk-sectioned rats(10). The latter authors, however, found that a marked infarction of the pituitary, similar to that seen in rat CR-1 of the present investigation, was produced by their technic of sectioning. Brolin did not state whether or not pituitary damage had been produced.

9. Brolin, S. E., *Acta Physiol. Scand.*, 1947, v14, 233.

10. Greep, R. O., and Barrnett, R. J.; Personal communication.

Summary. Bilateral, symmetrical, electrolytic lesions in the hypothalamus between the suprachiasmatic and caudal ventromedian nuclei prevented the goitrogenic response of the rat to thiouracil feeding. The iodide-concentrating capacity of the thyroid, however, increased to approximately the same extent as that of intact rats similarly treated. It appears that hypothalamic lesions of this type impair the secretion of thyrotrophin by the pituitary. It is not known whether this interference with thyrotrophin secretion is qualitative or quantitative, nor whether hypothalamic control is exerted by neural or hormonal mechanisms. The available evidence points to the secretion of a hypothalamic hormone, however.

I am indebted to Dr. E. B. Astwood for helpful advice and criticisms during the course of this investigation.

Received June 2, 1951. P.S.E.B.M., 1951, v77.

Subcutaneous Administration of Combined Fat Emulsion with Hyaluronidase.* (18863)

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Hyaluronidase has been proved to be an important adjuvant for the rapid absorption of a wide variety of fluids injected subcutaneously. The principles and the technics for the introduction of fluids into the body by means of hyaluronidase have been applied in the present study to the administration of a combined fat emulsion developed in our laboratory. This emulsion has been previously reported upon with regard to its clinical effects when infused intravenously into human subjects(1,2).

* Aided by a grant from the Abbott Research Laboratory.

1. Shafiroff, B. G. P., Mulholland, J. H., Roth, E., and Baron, H. C., PROC. SOC. EXP. BIOL. AND MED., 1949, v70, 343.

2. Shafiroff, B. G. P., and Mulholland, J. H., *Ann. Surg.*, 1951, v133, 145.

Method of study. Hyaluronidase and the combined fat emulsion were infused subcutaneously into 22 patients. Each subject received one liter of the 10% combined fat emulsion. The latter contained 100 g of purified coconut oil, 50 g of the amino acids contained in commercial intravenous protein hydrolysate solution, 50 g of dextrose, and 30 g of gelatin as the stabilizing agent. It provided approximately 1200 calories per liter and the particle size of the emulsion averaged 1 μ. Hyaluronidase was injected immediately prior to the infusion in doses of 500 units into the anterior aspect of each thigh about 6 inches above the knee. The emulsion was infused into the subdermal tissue at rates varying from 4 ml to 7 ml per minute. All studies were begun with each patient in the basal or postabsorptive state and continued after the