Table 2. Immunoquantitation of some isozymes of cytochrome P-450 in liver microsomes from normal and diabetic rats

	Cyt. P-450 UT-A (a)	Cyt. P-450 PB-B (b)	Cyt. P-450 ISF-G (a)	Cyt. P-450 BNF-B (a)
Control rats	1.58 ± 0.30	0.011 ± 0.005	0.18 ± 0.02	0.017 ± 0.003
Diabetic rats	0.23 ± 0.01	0.093 ± 0.016	0.11 ± 0.01	0.017 ± 0.009

For control and diabetic animals results are the mean ± SEM of 3 animals (a) or 7 animals (b). Each determination is made in duplicate. Results are expressed as nmol of cyt. P-450 × mg microsomal protein. In liver microsomes from control rats, the total of immunoquantitated isozymes exceeds that assayed by the spectrophotometric method. This is explained by the ability of the immunological method to detect also the corresponding apoprotein of the cytochrome P-450 isozyme assayed[7].

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0014-4754/86/101162-02$1.50 + 0.20/0

Intranuclear crystalloids in arcuate nucleus neurons after clomiphene citrate administration

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Summary. The action of high doses of clomiphene citrate on the nuclei of hypothalamic arcuate neurons of male cats has been studied. Clomiphene produces an accumulation of typical crystalloid material in the nuclei. After administration of a protein-synthesis inhibitor, no such material was observed in clomiphene-treated animals.
These ultrastructural features could possibly be due to a more intense protein synthesis in the hypothalamic arcuate neurons.
Key words. Hypothalamus; arcuate nucleus; intranuclear inclusions; clomiphene; male cat.

Nuclear inclusions in neurons have been described since the end of the 19th century[1, 9, 14, 20].
The first ultrastructural description of the so-called 'Intranuclear rodlets' of neurons was in 1964[26]. Subsequently, a number of papers treated this subject in areas other than the central and peripheral nervous system under normal, experimental and pathological conditions[2, 4, 10, 18, 19, 24, 25].
Clomiphene citrate has been successfully used to treat anovulatory states of varied etiology in women[13]. Although its exact mode of action has not been established, the final result seems to be the release of pituitary gonadotrophins, particularly luteinizing hormone (LH), and ovulation as a consequence. The regulation of LH-release is one of the likely functions of the arcuate nucleus (ARC) neurons.
The success of this pharmacological agent on spermatogenesis in infertile males varies considerably. Results seem to be dependent on dose, duration of treatment and previous degree of testicular alteration[8, 17].
We have excluded several possible causes of variability by using one species and sex (male cat), one specific substance (clomiphene citrate) and the injection into one (intraperitoneal) site of the animals.
Materials and methods. Twelve male cats (1.5–2.0 kg) were raised in our vivarium and housed under controlled environmental and feeding conditions. The following experimental groups were established:
a) Four cats were given a single i.p. injection of clomiphene citrate (250 mg/kg) dissolved in distilled water. These cats were sacrificed 24 h after the injection.
b) Two cats, treated with clomiphene citrate as described above were given a single i.v. injection of protein synthesis inhibitor, cycloheximide (20 mg/kg), 4 h before killing.
c) Two cats were given only cycloheximide as described above 4 h before killing.
d) Two cats were given a single i.p. injection of distilled water.
e) Two cats received no injections and were used as controls.
The animals were anesthetized with Nembutal (35 mg/kg) and

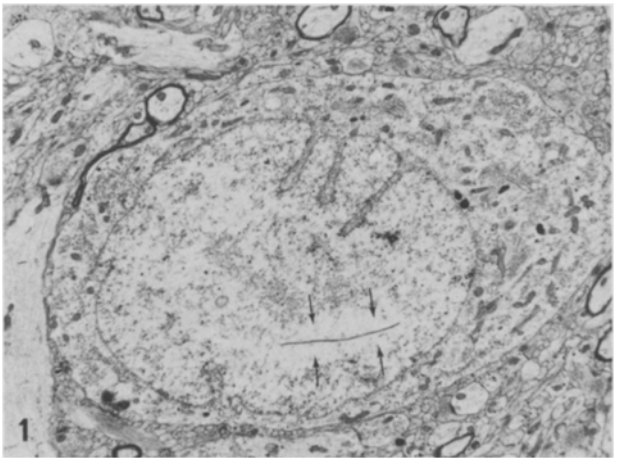

Figure 1. Arcuate nucleus neuron. Crystalloid intranuclear inclusion (arrows) (× 4900).

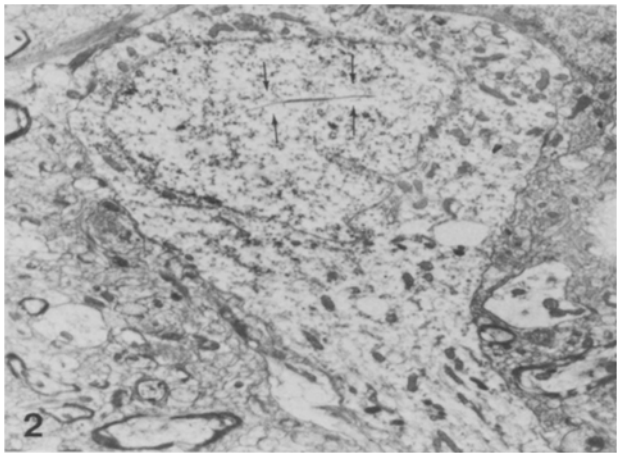

Figure 2. Intranuclear crystalloid (arrows) (× 4900).

perfused through the left cardiac ventricle with a mixture of 2.5% glutaraldehyde and 1% paraformaldehyde in 0.1 Na-cacodylate buffer (pH 7.5) at room temperature. All the animals had assisted respiration. After perfusion, coronal slices containing the middle part of the ARC and of the median eminence of the hypothalamus were cut, rostral to the infundibular stem. The pieces (about 0.5-mm thick) were immersed for 1 h in the fixative and then kept in the buffer for 12 h at 4 °C postfixed in 1% OsO_4 for 4 h at 4 °C, dehydrated in a graded series of acetones and embedded in epoxy resin d (Araldita, Fluka).

Ultrathin sections were obtained with a Reitchert OmU2 automatic microtome, mounted on copper grids and stained with uranyl acetate and lead citrate. Observations and photographs were obtained with a JEOL 100B electron microscope.

Results. Quantitative analysis. We studied 100 nuclei from each of the 12 animals. In the experimental group treated with clomiphene citrate, 191 (47.45%) ARC neurons presented a single nuclear crystalloid.

Intranuclear crystalloids were never observed in the ARC neurons of the control nor of the other experimental groups.

Ultrastructural study. The nuclear inclusions often showed up as single narrow structures surrounded by a clear halo. The halo and the density of the inclusion make its identification easy, by making the other morphological structures stand out (fig. 1). On other occasions, the nuclear inclusion displayed a thickening of variable width, and the clear halo was not evident (fig. 2). The type of inclusion found was a sheet (5–8 nm in diameter) formed by layers of filaments crossing at an angle of approximately 60°–70° (fig. 3).

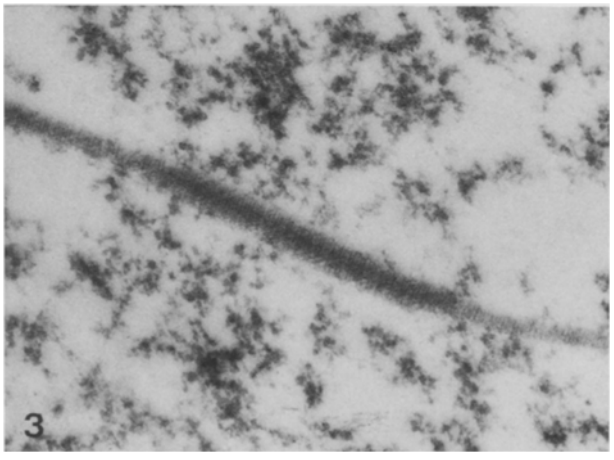

Figure 3. Higher magnification of the intranuclear inclusion of figure 2 (× 42,000).

Discussion. It is important to stress the fact that crystalloid nuclear inclusions are not common in neurons of the hypothalamic area. In other areas of the central and peripheral nervous system, frequency and location is variable from one site to another[11, 12, 18, 24, 25]. Several factors seem to be involved such as age[5], normal activity[3, 24] and experimental[21, 22] or pathological conditions[16, 27].

The formation of intranuclear crystalloids can be prevented by the action of the protein-synthesis inhibitor cycloheximide[22]. The occurrence of intranuclear crystalloids after clomiphene citrate administration may be due to an increased protein synthesis, since pretreatment with cycloheximide prevents the occurrence of these structures.

In a previous paper, we have pointed out that the specific ultrastructural changes observed in the rough endoplasmic reticulum could possibly indicate an increased protein synthesis in the cytoplasm of ARC neurons in male cats[6]. This synthesis was also prevented by the administration of cycloheximide.

All this seems to indicate that the relationship established between the formation of cytoplasmic and nuclear inclusions in other cells, could also be extended to neurons.

In male cats with high doses of clomiphene citrate, we have reported simultaneous atrophy of seminiferous tubules and the formation of crystalloid inclusions inside the RER cisterns of ARC neurons[7].

In men, doses of 400 mg/day suppress spermatogenesis because of damage to the basal membrane of the seminiferous tubules. However, doses between 100 and 200 mg/day do not seem to have any effect, and quantities below 50 mg/day induce spermatogenesis[8]. The reason for these different effects of different doses of the same pharmacological agent has not yet been found. It is evident that further studies are necessary to confirm whether there is any relationship between the events in ARC neurons and seminiferous tubules or if we must consider the possibility of them being different phases of the same process.

Acknowledgment. We would like to thank Prof. K. Fuxe for his helpful advice and suggestions in the planning of the experiments. We also thank P. Arribas for efficient technical help and R. W. Moyer for the English translation of the manuscript. This study was supported by grants from the Rodriguez Pascual Foundation, FIS and the Spanish Government CAICYT nr. 4461–79.

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0014-4754/86/101163-03$1.50 + 0.20/0

Fetal hypophysis as the main source of serum TSH in fetal rat

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Summary. Decapitation performed at days 17–18 leads to a drastic drop (82%) in blood TSH of 19 and 21-day-old rat fetuses below the mother's level. ^{125}I-TSH injected at 21 days into the mother's bloodstream is not found in fetal blood. The fetal hypophysis is the main source of fetal plasmatic TSH.
Key words. Rat fetal hypophysis; placental transfer.

TSH has been measured in the plasma of newborn rats and in 18–21-day-old fetuses[1–3]. Though it is generally accepted that the fetal thyroid pituitary axis develops independently of maternal TSH, the fetal hypophysis was not actually demonstrated to be the main source of the fetal serum TSH. In the literature, a few reports give indirect evidence of the probable lack of TSH transfer from the maternal blood to the fetus; propylthiouracil given to pregnant rats[4,5] or rabbit[6] induces a hypertrophy of the fetal thyroid in normal but not in decapitated fetuses; this indicates that maternal TSH does not replace fetal hormone for the fetal goiter induction. The injection of TSH into pregnant rats enhances formation of colloid droplets in the thyroid of the mother but not in that of the fetus, whereas increased fetal serum TSH stimulates the appearance of colloid droplets in the fetal thyroid[7]. To our knowledge, transplacental permeation of radioactive-labeled TSH has been studied only in sheep[8,9], where no transfer was found in either direction, i.e. mother to fetus or fetus to mother. However it cannot be automatically assumed that such a transfer does not occur in the rat because of the hemochorial placenta of this species; only 3 tissue layers separate the maternal and fetal circulations, instead of 6 layers in the epitheliochorial sheep placenta[10]. TSH might permeate the rat placenta more easily than the sheep placenta. This study was designed in an attempt to trace the origin of the fetal TSH in the rat; firstly, the effects of the suppression of the activity of the fetal hypophysis by decapitation on blood TSH level, and secondly, the transfer, to the fetus, of ^{125}I-TSH injected in the mother were studied.

Material and methods. Pregnant Wistar rats purchased from a breeding center (IFFA-CREDO, l'Arbresle, 69210 France) were used in these experiments. The mating day, determined by the presence of spermatozoa in the vaginal smear, was regarded as day 0 of gestation. On their arrival at the laboratory, the animals were housed at 23 °C and fed ad libitum on UAR commercial diet, weekly supplemented with salad and bread.

During all surgical procedures mothers were anesthetized with ether. In some litters 3 or 4 fetuses were totally decapitated at day 17 or 18 (section at the neck level) according to the technique of Jost[11–12]. At 19 or 21 days blood samples were taken from the mother and from both decapitated and unoperated fetuses. Utero-placental circulation was not interrupted during sampling[13]. Samples were collected on EDTA and centrifuged at 0–4 °C. The plasma fraction was immediately frozen pending assay. Operated animals were separately caged in a quiet room.

Plasma TSH was measured using the radioimmunoassay reagents of the NIADDK rat pituitary program. TSH iodination was achieved using ^{125}I (Amersham, England) and iodogen (Pierce, Chem. Co., USA) according to the technique of Salicinski et al.[13]. Results are expressed as NIAMDD-r-TSH-RP1. The detection limit of the assay was 6 ng/ml; the percentage of recovery of added amounts of TSH was 103 ± 2%.

In another experiment, 10^7 to 1.5×10^7 cpm of ^{125}I- TSH solution were injected at day 21 into the blood of the anesthetized mother via the carotid. At various times between 15 and 180 min, blood samples were withdrawn from the mother's carotid and from 2 or 3 fetuses. The total radioactivity of the samples including both ^{125}I-TSH and iodine resulting from TSH catabolism was measured. The ^{125} I-TSH fraction was ascertained by recognition with an anti-TSH-antiserum (NIAMDDDK anti-TSH-S5) and by immune complex precipitation with a second antibody (preliminary experiments were carried out to determine the amount of antibodies required to precipitate 80% of a 10^5 to 1.5×10^5 cpm aliquot of TSH solution). The animals were quickly killed at the end of the blood sampling before recovery from the anesthesia.

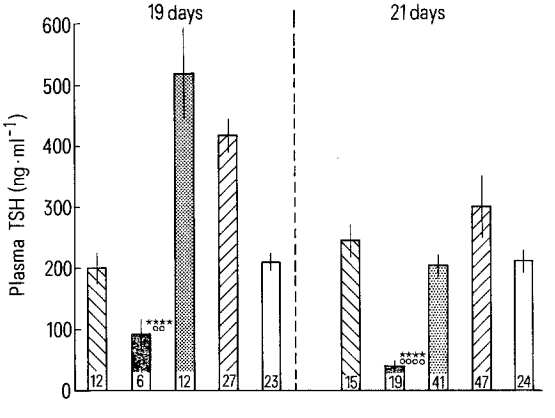

Figure 1. Plasma TSH at day 19 and 21 in normal rat fetuses, their unoperated mothers, in fetuses decapitated at days 17 or 18, unoperated littermate fetuses, and laparotomized mothers. The number of measurements is indicated at the foot of each column. Standard deviations are represented by vertical bars. Statistical comparison was made between decapitated fetuses and their mothers: ○○, $p < 0.02$; ○○○○, $p < 0.001$ and between decapitated fetuses and their unoperated littermates: ****, $p < 0.001$.