From our data we cannot determine whether the variation described is due to a variation in female receptivity and male virility of *D. melanogaster* and *D. simulans* respectively, or whether it is related to variation in the sexual discriminative specific behavior, that is, sexual isolation. This problem is at present under study.

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Comparison of the in vitro development of mouse single blastomeres with and without the zona pellucida

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Summary. No difference was observed during in vitro development between mouse single blastomeres with and without the zona pellucida, isolated from 2- and 4-cell stage eggs.
Key words. Mouse blastomeres; blastomeres, mouse; zona pellucida.

The developmental potential of individual blastomeres from cleaving eggs has been extensively studied in mammals. Two major methods have been employed for obtaining blastomeres; the destruction of sister blastomeres inside the zona pellucida[1–4], or isolation from each other after removal of the zona[5,6]. Each method has its advantages and disadvantages. The latter gives rise to naked blastomeres. The zona pellucida is necessary for the transit of the cleaving-stage egg through the oviduct in normal pregnancy[7,8]. However, the role of the zona in the development of blastomeres in vitro is little understood. We have recently developed an miproved method[9] for isolating mouse blastomeres by electrically destroying other blastomeres inside the zona. The present report describes the in vitro development of single mouse blastomeres with and without the zona pellucida.

Materials and methods. Cleaving eggs for preparation of single blastomeres were collected by flushing oviducts excised from C57Bl/crSlc females, which were injected with 5–10 i.u. of pregnant mare serum gonadotropin followed by the same dose of human chorionic gonadotropin (HCG) 48 h later and were mated with fertile DBA/2Slc males. Two-cell stage eggs were collected at 38–40 h, and 4-cell stage eggs at 48–50 h after the HCG injection. Single blastomeres with the zona were obtained by electrically destroying all blastomeres except one inside the zona as follows. The eggs were placed in a drop of the culture medium[10] under paraffin oil in a special dish[9]. While the egg was held by suction on the tip of the holding pipet connected to a micromanipulator, a glass microelectrode was gently pushed through the zona and was introduced into the unwanted blastomere, which was then charged by a direct current (60 μA, 8 V). The target blastomere disintegrated completely in a few seconds after the charge. Micromanipulation was carried out at room temperature and at a magnification of 200× under Nomarski optics.

Blastomeres without the zona were isolated as follows; the zona was removed by a 20–50-sec incubation with warmed (37 °C) acidic Tyrode's solution (pH 2.0)[11] and the zona-free embryo was separated mechanically into individual blastomeres with a fine-bore glass pipet in phosphate-buffered saline solution containing 0.02% EDTA and 0.2% trypsin. The blastomeres that were isolated by the two methods were incubated in drops of culture medium covered with paraffin oil at 37 °C in an atmosphere of 5% CO_2, 95% air.

Results and discussion. The table shows the in vitro development of various mouse embryos with and without the zona pellucida. The naked one-half embryos (½-embryos) isolated

In vitro development of single mouse blastomeres with and without the zona pellucida isolated from 2- and 4-cell stage eggs

With or without the zona	Sorts of embryos	No. of embryos	Embryonic structures at blastocyst stage: Blastocyst	Morula	Trophoblastic vesicle	Non-integrated form	Degenerated or arrested
With the zona	2-cell egg	62	59 (95.2)				3 (4.8)
	4-cell egg	66	61 (92.4)				5 (8.6)
	½-embryo	104	88 (84.6)	2 (1.9)	3 (2.9)	2 (1.9)	9 (8.7)
	¼-embryo	116	41 (35.3)	21 (18.1)	24 (20.7)	16 (13.8)	14 (12.1)
Without the zona	2-cell egg	78	71 (91.0)			3 (3.8)	4 (5.1)
	4-cell egg	67	65 (97.0)				2 (3.0)
	½-embryo	205	180 (87.8)	7 (3.4)	5 (2.4)	1 (0.5)	18 (8.8)
	¼-embryo	87	37 (42.5)		27 (31.0)	9 (10.3)	7 (8.1)

Values were obtained from 4 to 6 experiments. Values in parentheses are percentages of the number of embryos used.

from 2-cell stage eggs developed in vitro, as well as the zona-intact ones, into normal blastocysts at the expected time. The size of blastocysts formed from these embryos was similar to or slightly smaller than that of normal blastocysts. By contrast, both the one-quarter embryos (¼-embryos) with and without the zona showed a marked drop of developmental potential, and formed various abnormal embryos at the blastocyst stage, which were similar to those described in a previous report[12]. The lack of developmental potential of ¼-embryos might be due to the inadequacy of the number of cells composing the embryos developed from ¼-embryos, as Rossant[13] has suggested; the volume of most blastocysts from ¼-embryos was far smaller than that of ordinary ones. Kelly[14] reported that ¼- and ⅛-embryos of mouse could give rise to embryos which could be born normally as chimaeras when combined with other blastomeres to restore the normal cell numbers.

The rate of development into blastocysts was slightly lower in the zone-intact blastomeres than the zona-free ones. The debris of destroyed blastomeres remaining inside the zona might affect the development of the intact blastomeres.

It was reported that the zona-free embryos did not undergo any further cleavage when transferred to the oviduct because they adhered to the wall of the oviduct[7,8]. When naked embryos were inserted into an empty zona or embedded in agar and transferred to recipient oviducts, they produced live young[6,15]. On the other hand, naked morulae or blastocysts which were transferred into the uterus gave rise to normal conception[16]. However, the possible significance of the zona in normal development in vitro of single blastomeres is not well known. In this experiment, there was no difference in developmental potential in vitro between blastomeres with and without the zona. We therefore conclude that the presence of the zona pellucida is not essential for the development of single mouse blastomeres in vitro.

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Neuron numbers in hypothalamic nuclei of young, middle-aged and aged male rats

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Summary. Morphologic analysis of nine hypothalamic areas revealed significant decreases in the number of neurons per unit area in the ventral medial and arcuate nuclei. These data suggest that altered neuron numbers in the VMW and perhaps the ARC may participate in the well documented reductions in endocrine and neuroendocrine function observed in aging rats.
Key words. Hypothalamus; neuron number; aging; neuroendocrinology.

Several studies have suggested that morphologic alterations in the hypothalamus occur with advancing age. Glees et al.[3] found degenerative changes in mitochondria in the hypothalamus of aging monkeys. Age-related changes, which included a deterioration and loss of dendritic surfaces, were also found in hypothalamic neurosecretory nuclei of old mice[4]. Detailed studies of the arcuate nucleus (ARC) of the hypothalamus have shown a decrease in axosomatic and axo-dendritic synapses in old male rats[5]. There is a loss of neurons in the medial preoptic area (mPOA), anterior hypothalamic area (AHA) and the ARC in aging female rats[6]. Each of these studies provides morphologic data which can be correlated with well documented age-related physiological changes in endocrine and neuroendocrine function[7,8].

Aging male Sprague-Dawley rats are often used to study a wide variety of endocrine and neuroendocrine dysfunctions during senescence[9–12]. Since there are relatively few reports concerning any morphological changes in the hypothalamus of this animal model, it became imperative to determine whether changes in neuron number occurred in specific hypothalamic nuclei of aging male Sprague-Dawley rats.

Materials and methods. Male Sprague-Dawley rats, ages 3, 12 and 24 months (young, middle-aged and aged, respectively), were obtained from Charles River Breeding Laboratories (Wilmington, MA). These rats were Cesarian derived and raised behind specific pathogen free barriers. Animals were housed five per cage and provided food and water ad libitum in a temperature (24 °C) and photoperiod controlled (12 h light – 12 h dark, lights on at 06.00 h) animal room. Normal aging pathology and requirements for short-term handling have been reported elsewhere[9,13].

The brains of five young, five middle-aged and six aged rats were removed following decapitation and fixed in 10% neutral buffered formalin, dehydrated and embedded in paraffin. The hypothalami were sectioned at 9 µm and stained with cresyl violet. The mPOA, suprachiasmatic nucleus (SCN), paraventricular nucleus (PVN), ventral medial nucleus (VMN), dorsal medial nucleus (DMN), ARC, subventricular portion of the ARC (SV) and the medial mammillary nucleus (MM) were studied according to the following procedure which was previously reported[14]. The numbers of neurons in these nuclei were counted in every fifth section under an AO microscope